Methods of inhibiting Alu RNA and Therapeutic Uses Thereof

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METHODS OF INHIBITING ALU RNA AND THERAPEUTIC USES THEREOF

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None

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ABSTRACT
The presently-disclosed subject matter includes methods of identifying an Alu RNA inhibitor, and methods and compositions for inhibiting Alu RNA. Methods and compositions can be used for the treatment of geographic atrophy and other conditions of interest.

2 Claims, 25 Drawing Sheets
References Cited

OTHER PUBLICATIONS


* cited by examiner
FIG. 1

(a) Fold change in mRNA

(b) Fold change in DICER1 (protein)

(c) Normal Geographic atrophy

(d) BEST1 Cre; Dicer1

(e) RF chc

(f) Control

DICER1, DROSHA, DGCR8, AGO2

Normal GA

* NS
FIG. 1, Continued
FIG. 2
FIG. 3
FIG. 4
FIG. 5
FIG. 6
FIG. 7

a. Fold change in DICER1 RNA

b. DICER1
c. isotype

GA

Normal

FIG. 7
DICER1

Best disease

Retinal detachment

Retinitis pigmentosa

![Graph showing fold change in Dicer1 mRNA](FIG. 8)
AAV1-BEST1-Cre

wild-type    wild-type

wild-type    Dicer1<sup>−/−</sup>

FIG. 9
FIG. 12

FIG. 13

Cell viability (%)
FIG. 14
FIG. 18

Nucleus Cytoplasm

DICER1

Tubulin
FIG. 19

Fold change

Normal

GA

DICER1 as

Ctrl as

L1.3  HERV-WE1  hY3

FIG. 19
**FIG. 20**

- **a**
  - Fold change in Alu RNA
  - Control (Ctrl) vs. DICER1

- **b**
  - Alu NB: 300nt
  - U6 NB
  - GA Normal

- **c**
  - Fold change (%)
  - ADAR2, NicN, NLPR, SLFNT1
  - Ctrl vs. DICER1

**FIG. 21**

- **a**
  - Fold change in YSL RNA
  - Normal vs. GA

- **b**
  - Fold change in YSL RNA
  - Ctrl vs. DICER1

- **c**
  - Fold change in YSL RNA
  - Ctrl vs. Alu
FIG. 23

Fold change in Alu RNA

FIG. 24

a) Human GA csRNA, Vehicle

b) DICER1-cleaved Human GA Alu csRNA, Mock DICER1-cleaved Human GA Alu csRNA

FIG. 25

p7SL, tRNA, pmiR29b1, pmiR26a2
FIG. 28

(a) Bar graph showing human RPE cell viability (%). The graph compares the viability of cells with and without the presence of Alu Frag: pNull, pAlu, and pAlu + Alu Frag. The graph indicates no significant difference (NS) between the groups.

(b) Images depicting Alu Frag and Alu Frag + pAlu conditions. The images show cellular responses under these conditions.
METHODS OF INHIBITING ALU RNA AND THERAPEUTIC USES THEREOF

RELATED APPLICATIONS

This application is a divisional of U.S. patent application Ser. No. 13/701,450, now allowed, which is a 371 application of International Patent Application No. PCT/US2011/038753, filed Jun. 1, 2011, which claims priority from U.S. Provisional Application Ser. No. 61/396,747, filed on Jun. 1, 2010; U.S. Provisional Application Ser. No. 61/432,110, filed Jan. 12, 2011; and U.S. Provisional Application Ser. No. 61/432,948, filed Jan. 14, 2011. The entire disclosures of these applications are incorporated herein by this reference.

TECHNICAL FIELD

The presently-disclosed subject matter relates to uses of DICER overexpression and the inhibition of Alu RNA.

INTRODUCTION

Geographic atrophy, an advanced form of age-related macular degeneration that causes blindness in millions of people worldwide and for which there is no approved treatment, results from death of retinal pigmented epithelium (RPE) cells. As described herein the present inventors show that expression of DICER, an enzyme involved in microRNA (miRNA) biogenesis, is reduced in the RPE of human eyes with geographic atrophy, and that conditional ablation of Dicer1 induces RPE degeneration in mice. Surprisingly, ablation of seven other enzymes responsible for miRNA biogenesis or function does not induce such pathology. Instead, knockdown of Dicer1 leads to accumulation of Alu repeat RNA in human RPE cells and of B1 and B2 (Alu-like elements) repeat RNAs in the RPE of mice.

Alu RNA is dramatically increased in the RPE of human eyes with geographic atrophy, and introduction of this pathological RNA induces death of human RPE cells and RPE degeneration in mice. Antisense oligonucleotides targeting Alu/B1/B2 RNAs inhibit DICER1 depletion-induced RPE degeneration despite persistence of global miRNA downregulation. DICER1 degrades Alu RNA, and Alu RNA loses the ability to induce RPE degeneration in mice when digested by DICER1. These findings reveal a novel miRNA-independent cell survival function for DICER1 via degradation of retrotransposon transcripts, introduce the concept that Alu RNA can directly cause human pathology, and identify new molecular targets for treating a major cause of blindness.

Age-related macular degeneration (AMD), which is as prevalent as cancer in industrialized countries, is a leading cause of blindness worldwide. In contrast to the neovascular form of AMD, for which many approved treatments exist, the far more common atrophic form of AMD remains poorly understood and without effective clinical intervention. Extensive atrophy of the retinal pigment epithelium (RPE) leads to severe vision loss and is termed geographic atrophy, the pathogenesis of which is unclear. As described herein, the present inventors identify dysregulation of the RNase DICER1 and the resulting accumulation of transcripts of Alu elements, the most common small interspersed repetitive elements in the human genome, as a cause of geographic atrophy, and describe treatment strategies to inhibit this pathology in vivo.

SUMMARY

The presently-disclosed subject matter meets some or all of the needs identified herein, as will become evident to those of ordinary skill in the art after a study of information provided in this document.

This Summary describes several embodiments of the presently-disclosed subject matter, and in many cases lists variations and permutations of these embodiments. This Summary is merely exemplary of the numerous and varied embodiments. Mention of one or more representative features of a given embodiment is likewise exemplary. Such an embodiment can typically exist with or without the feature(s) mentioned; likewise, those features can be applied to other embodiments of the presently-disclosed subject matter, whether listed in this Summary or not. To avoid excessive repetition, this Summary does not list or suggest all possible combinations of such features.

In some embodiments, the presently-disclosed subject matter includes a method of treating a condition of interest, including inhibiting Alu RNA associated with a cell of a subject. In some embodiments, the condition of interest is selected from: geographic atrophy, dry age-related macular degeneration, thalassemia, familial hypercholesterolemia, Dent's disease, acute intermittent porphyria, anterior pituitary aplasia, Apert syndrome, Hemophilia A, Hemophilia B, glycerol kinase deficiency, autoimmune lymphoproliferative syndrome, X-linked agammaglobulinemia, X-linked severe combined immunodeficiency, adrenoleukodystrophy, Menkes disease, hyper-immunoglobulin M syndrome, retinal blinding, Type 1 anti-thrombin deficiency, Muckle-Wells syndrome, hypocalciuric hypercalcemia and hyperparathyroidism, cholinesterase deficiency, hereditary desmoid disease, chronic hemolytic anemia, cystic fibrosis, branchioto-renal syndrome, lipoprotein lipase deficiency, CHARGE syndrome, Walker Warburg syndrome, Complement deficiency, Musculopidosis type II, Breast cancer, ovarian cancer, prostate cancer, von Hippel Lindau disease, Hereditary non-polyposis colorectal cancer, multiple endocrine neoplasia type 1, hereditary diffuse gastric cancer, hepatoma, neurofibromatosis type 1, acute myeloid leukemia, T-acute lymphoblastic leukemia, and Ewing sarcoma.

In some embodiments, the presently-disclosed subject matter includes a method of treating geographic atrophy, including inhibiting Alu RNA associated with an RPE cell.

In some embodiments, the presently-disclosed subject matter includes a method of protecting an RPE cell, including inhibiting Alu RNA associated with the RPE cell.

In some embodiments, the RPE cell is of a subject having age-related Alu RNA is dramatically increased in the RPE of human eyes with geographic atrophy, and introduction of this pathological RNA induces death of human RPE cells and RPE degeneration in mice. Antisense oligonucleotides targeting Alu/B1/B2 RNAs inhibit DICER1 depletion-induced RPE degeneration despite persistence of global miRNA downregulation. DICER1 degrades Alu RNA, and Alu RNA loses the ability to induce RPE degeneration in mice when digested by DICER1. These findings reveal a novel miRNA-independent cell survival function for DICER1 via degradation of retrotransposon transcripts, introduce the concept that Alu RNA can directly cause human pathology, and identify new molecular targets for treating a major cause of blindness.

Age-related macular degeneration (AMD), which is as prevalent as cancer in industrialized countries, is a leading cause of blindness worldwide. In contrast to the neovascular form of AMD, for which many approved treatments exist, the far more common atrophic form of AMD remains poorly understood and without effective clinical intervention. Extensive atrophy of the retinal pigment epithelium (RPE) leads to severe vision loss and is termed geographic atrophy, the pathogenesis of which is unclear. As described herein, the present inventors identify dysregulation of the RNase DICER1 and the resulting accumulation of transcripts of Alu elements, the most common small interspersed repetitive elements in the human genome, as a cause of geographic atrophy, and describe treatment strategies to inhibit this pathology in vivo.

In some embodiments, the presently-disclosed subject matter relates to uses of DICER overexpression and the inhibition of Alu RNA.
embodiments, increasing levels of a DICER polypeptide comprises overexpressing the DICER polypeptide in the cells. In some embodiments, increasing levels of a DICER polypeptide comprises using a vector comprising a nucleotide encoding the DICER polypeptide. In some embodiments, the vector is a viral vector. In some embodiments, the virus is selected from an adeno-associated virus, a lentivirus, and an adeno virus. In some embodiments, the vector is a plasmid vector. In some embodiments, the nucleotide encoding the DICER polypeptide is selected from SEQ ID NO: 9 and SEQ ID NO: 8. In some embodiments, the DICER polypeptide is selected from SEQ ID NO: 9, 10, 11, 12, 13, 14, 15, 16, 18, and 20. In some embodiments, the DICER polypeptide comprises a functional fragment of the sequence of SEQ ID NO: 9, 18, or 20. In some embodiments, the DICER polypeptide comprises the following amino acid residues of the polypeptide of SEQ ID NO: 9: 605-1922, 605-1912, 1666-1922, 1666-1912, 605-1786 and 1800-1922, 605-1786 and 1800-1912, 1666-1786 and 1800-1922, 1666-1786 and 1800-1912, 1276-1922, 1276-1912, 1276-1786 and 1800-1922, 1276-1786, 800-1912, 1275-1824, or 1276-1824.

In some embodiments of the methods of the presently disclosed subject matter including inhibiting Alu RNA associated with a cell, the inhibiting Alu RNA comprises increasing levels of a DICER polypeptide comprises using DICER mRNA or a functional fragment thereof. In some embodiments, the DICER mRNA has the sequence of SEQ ID NO: 17, 19, or 21. In some embodiments, the DICER mRNA encodes a DICER polypeptide, for example, the DICER polypeptide of SEQ ID NO: 9, 18, or 20, or a functional fragment thereof.

In some embodiments of the methods of the presently disclosed subject matter including inhibiting Alu RNA associated with a cell, the inhibiting Alu RNA comprises administering an oligonucleotide targeting Alu RNA. In some embodiments, the oligonucleotide has a sequence including a sequence selected from SEQ ID NO: 22, 23, 24, 25, and 26. In some embodiments, at least two oligonucleotides are administered. The presently-disclosed subject matter further includes an isolated oligonucleotide that inhibits the expression of Alu RNA, including a sequence selected from SEQ ID NO: 22, 23, 24, 25, and 26 and including about 29 to 100 nucleotides.

In some embodiments of the methods of the presently disclosed subject matter including inhibiting Alu RNA associated with a cell, the inhibiting Alu RNA comprises administering an siRNA targeting Alu RNA. In some embodiments, the siRNA includes a first strand having a sequence selected from SEQ ID NO: 1, 2, 3, 4, 5, and 6. The presently-disclosed subject matter further includes an isolated double-stranded RNA molecule that inhibits expression of Alu RNA, wherein a first strand of the double-stranded RNA comprises a sequence selected from SEQ ID NO: 1, 2, 3, 4, 5, and 6 and including about 19 to 25 nucleotides.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 DICER1 deficit in geographic atrophy induces RPE degeneration. a, DICER1 mRNA abundance, relative to 18S rRNA, monitored by real-time RT-PCR, was lower in the retinal pigmented epithelium (RPE) of human eyes with geographic atrophy (GA; n=10) compared to the RPE of normal human eyes without GA (n=11). P<0.004 by Mann Whitney U test. The abundance of DROSHA, DGCIR, and EIF2C2 (encoding AGO2) mRNA transcripts in the RPE was not significantly different (P>0.11 by Mann Whitney U test) in human eyes with geographic atrophy and control eyes. Transcript abundance quantified by real-time RT-PCR and normalized to 18S rRNA and to control eye levels. n=10-11. b, Relative quantification of DICER1 protein abundance, relative to Vinculin, assessed by Western blotting (Supplementary FIG. 1), was lower in the RPE of human eyes with geographic atrophy (GA; n=4) compared to the RPE of normal human eyes without GA (n=4). P=0.005 by Student t test. c, Immunohistochemistry for DICER1 (blue) showed reduced protein abundance in the RPE of human eyes with GA compared to normal eyes without GA. d, Fundus photographs show extensive RPE degeneration in BESTI Cre; Dicer1/2 mice but not in littermate control mice. e, Toluidine blue-stained sections show marked RPE degeneration in BESTI Cre; Dicer1/2 mice compared to normal RPE and choroid. Arrowheads point to basal surface of RPE. f, Flat mounts of the RPE and choroid stained with antibodies against zonula occludens-1 (ZO-1; red) show marked disruption of the RPE monolayer architecture in BESTI Cre; Dicer1/2 mice compared to the uniformly tesselated RPE layer in littermate control mice. g, Fundus photographs show RPE degeneration in Dicer1/2 mice following subretinal injection of AAV1-BEST1-Cre but not AAV1-BEST1-GFP. h, Toluidine blue-stained sections show marked degeneration of RPE and photoreceptor outer segments in Dicer1/2 mice following subretinal injection of AAV1-BEST1-Cre but not AAV1-BEST1-GFP. i, Flat mounts show marked increase in RPE cell size and distortion of RPE cell shape in Dicer1/2 mice following subretinal injection of AAV1-BEST1-Cre but not AAV1-BEST1-GFP. RPE cell borders outlined by ZO-1 staining (red). Nuclei stained blue with Hoechst 33342. Representative images shown. n=5-13 (d-l); 10-12 (g-i). Scale bars, (c,e,h). 10 μm; (f,i) 20 μm. j, Transfection of adenoviral vector coding for Cre recombinase (Ad-Cre) in RPE cells isolated from Dicer1/2 mice resulted in loss of cell viability, as monitored by MTS assay at 7 days, compared to transfection with Ad-Null or untreated (no Tx) cells. k, Transfection of antisense oligonucleotide (as) targeting DICER1 into human RPE cells resulted in increasing loss of cell viability over time compared to scrambled sequence antisense (Ctrl as)-treated cells. n=6-8.

FIG. 2 Alu RNA accumulation in geographic atrophy triggered by DICER reduction. a, Immunohistochemistry with anti-double stranded RNA (dsRNA) antibody (J2) shows abundant accumulation of dsRNA (blue staining) in the retinal pigmented epithelium (RPE) of a human eye with geographic atrophy. b, Lack of immunolabeling with an isotype antibody in the same eye with geographic atrophy confirms specificity of dsRNA staining. c,d, dsRNA is immunolocalized (blue staining) in the RPE and sub-RPE deposits (drusen) of a human eye with geographic atrophy (c) but not in the RPE of a normal (control) eye (d). Scale bars, (a-d), 10 μm. n=10 (a-d) e, PCR amplification of dsRNA immunoprecipitated by J2 antibody from RPE isolates from human eyes with geographic atrophy and normal eyes yielded amplicons with sequence homology to Alu sequences (Supplementary FIG. S7) in eyes with geographic atrophy but not in normal eyes. Water negative control (−) showed no amplification and positive control (+) recombinant dsRNA showed predicted amplicon. f, Alu RNA abundance, relative to 18S rRNA, monitored by real-time RT-PCR, was higher in the RPE of human eyes with geographic atrophy compared to the RPE of normal human eyes without GA (n=7). P<0.05 by Student t test. There was no significant

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difference in Alu RNA abundance in the neural retina of these two patient groups. Values normalized to relative abundance in normal eyes.

FIG. 3 DICER1 degrades Alu RNA. a, Transfection of antisense oligonucleotide (as) targeting DICER1 into human RPE cells induced a time-dependent increase in the abundance of Alu RNA transcripts. b, c, Transfection of adenoviral vector coding for Cre recombinase (Ad-Cre) into mouse RPE cells isolated from Dicerflf mice increased, in the nucleus (b) and the cytoplasm (c), the abundance of B1 and B2 RNAs, the Alu-like repetitive elements in the mouse, compared to cells transfected with adenoviral vector coding for green fluorescent protein (Ad-GFP). d, DICER1 as treatment of human RPE cells upregulated Alu RNA levels in both the nucleus (Nuc) and cytoplasm (Cyt). e, Alu RNA isolated and cloned from the RPE of human eye with geographic atrophy was degraded by recombinant DICER1 digestion (+) as visualized by agarose gel electrophoresis. Digestion with heat denatured DICER1 did not degrade Alu RNA. Image representative of 6 experiments. f, The increased abundance of Alu RNA in human RPE cells transfected with plasmid coding for Alu (pAlu) compared to pNull or no treatment (no Tx) at 24 h was reduced by co-transfection with pDICER1. *P<0.05, n=4–8 (a–d, f). RNA abundance was quantified by real-time RT-PCR, normalized to 18S rRNA levels, and normalized to levels in control as-treated (for Alu) or Ad-GFP-infected cells (for B elements).

FIG. 4 DICER1 protects RPE cells from Alu RNA cytotoxicity. a, Transfection of mouse or human retinal pigmented epithelium cells (mRPE or hRPE) with plasmid coding for Alu RNA (pAlu) compromised cell viability. b, Subretinal administration of pAlu induced RPE degeneration in wild-type mice whereas pNull did not do so. Fundus photograph (top row) shows area of degeneration in pAlu injected eye compared to the normal appearance in pNull. Flat mount preparations stained with anti-zonula occludens-1 antibody (ZO-1, red, bottom row) show marked distortion of RPE cell shape and size compared to pNull-injected eye. c, Alu RNA induced dose-dependent increase in cell death of human RPE cells. d, Cell death of human RPE cells induced by transfection of pAlu was inhibited by co-transfection with pDICER1 but not pNull. (a, c, d) Cell viability monitored by MTS assay at 2 days. Values normalized to null plasmid (pNull) transfected or vehicle (Ctrl) as. *P<0.05 by Student t test. n=4–6. e, f, Subretinal co-administration of pDICER1, but not pNull, inhibited pAlu induced RPE degeneration in wild-type mice. f, Subretinal administration of Alu RNA isolated and cloned from the RPE of a human eye with geographic atrophy (GA) induced RPE degeneration in wild-type mice whereas subretinal injection of vehicle did not. g, Subretinal injection of this Alu RNA, when subjected to cleavage by DICER1, did not induce RPE degeneration in wild-type mice whereas Alu RNA subjected to mock cleavage by DICER1 did do so, as evident on fundus photography (top row) or flat mount preparation (bottom row). Area of degeneration outlined by blue arrowheads in fundus photographs (b, e–g). Scale bars (20 μm). n=10–15 (b, e–g).

FIG. 5 DICER1 dysregulation induces RPE cell death via Alu RNA accumulation. a, Loss of human RPE cell viability, as monitored by MTS assay, induced by transfection of antisense oligonucleotide (as) targeting DICER1 was rescued by co-transfection of Alu RNA as. Levels normalized or compared to transfection with control (Ctrl) antisense oligonucleotide. b, Alu RNA as induced accumulation of Alu RNA induced by DICER1 as, c, Ad-Cre but not Ad-Null induced loss of cell viability of Dicerflf mouse RPE cells. This was rescued by transfection of antisense oligonucleotide targeting B1 and B2 RNAs but not by control (Ctrl) antisense oligonucleotide. Levels normalized to untreated cells (no Tx), d, B1/B2 RNA as inhibited accumulation of B1 and B2 RNAs induced by Ad-Cre-induced Dicer1 depletion. *P<0.05 by Student t test. n=4–6 (a–d). d, Subretinal AAV-BEST1-Cre administration induced RPE degeneration (blue arrowheads in fundus photograph on top row and marked increase in RPE cell size and distortion of RPE cell shape in ZO-1 stained (red) RPE flat mounts (bottom row) in Dicerflf mice 20 days after injection. Subretinal administration of cholesterol-conjugated B1/B2 as, but not Ctrl as, 10 days after AAV-BEST1-Cre injection inhibited RPE degeneration (e) and abundance of B1/B2 RNAs in the RPE of these mice, as monitored by real-time RT-PCR at 10 days after as injection, normalized to 18S rRNA levels, and normalized to levels in eyes treated with cholesterol-conjugated Ctrl as (f). n=8 (e, f). Scale bar, 20 μm. (e, g, DICER1 as treatment of human RPE cells led to global reduction of miRNA expression at 2 days compared to Ctrl as. There was no significant difference in miRNA abundance between Alu as and Ctrl as-treated Dicer1 depleted cells. n=3.

FIG. 6 DICER1 levels in RPE are reduced in geographic atrophy. Western blots of macular RPE lysates from individual human donor eyes show that DICER1 protein abundance, normalized to the levels of the housekeeping protein Vinculin, are reduced in geographic atrophy (GA) compared to age-similar control human eyes without age-related macular degeneration.

FIG. 7 DICER1 levels in neural retina are unchanged in geographic atrophy. a, DICER1 in RPE are reduced in geographic atrophy. a, DICER1 mRNA abundance in the neural retina, as monitored by real-time RT-PCR, was not significantly different (P>0.05 by Mann Whitney U test) between normal human retinas and those with geographic atrophy. Levels normalized to 18S rRNA abundance and to normal retinas. n=7–8 (b–e). DICER1 protein immunolocalization in the neural retina was not different between human eyes with geographic atrophy (b) and normal (d) eyes. Specificity of DICER1 staining was confirmed by absence of reaction production with isotype control antibody (c, e). Representative images shown. n=8. Scale bars (20 μm, b–e).

FIG. 8 DICER1 is not generically downregulated in retinal diseases. Immunolocalization studies revealed abundant DICER1 protein expression (blue, left column) in the RPE in the eye of an 85-year-old man with Best disease (vitriform macular dystrophy), a 66-year-old man with retinal detachment secondary to choroidal melanoma, and a 72-year-old woman with retinitis pigmentosa. Specificity of DICER1 staining was confirmed by absence of reaction production with isotype control antibody (right column). Representative images shown. n=13. Scale bars (10 μm). DICER1 mRNA expression in the RPE was not significantly (NS) different in Ccl2−/−Ccr2−/− mice or Cpp−/−Hepl−/− mice compared to their background strains. Transcript abundance quantified by real-time RT-PCR and normalized to 18S rRNA and to control eye levels. n=6. NS, not significant.

FIG. 9 Cre recombinase expression does not induce retinal pigmented epithelium (RPE) degeneration. Subretinal administration of adeno-associated viral vector coding for Cre recombinase directed by the BEST1 promoter (AAV1-BEST1-Cre) in wild-type mice did not induce retinal toxicity that was evident on fundus photography (top left) and did not disrupt the tiling pattern of the RPE monolayer (top right). Circular flash artifact is seen in the centre of the fundus photograph. RPE cell borders delineated by staining with anti-ZO-1 antibody (red) and nuclei stained by Hoechst
FIG. 10 Retinal pigmented epithelium (RPE) cell dysmorphism in human age-related macular degeneration eye with atrophy. In contrast to the well tessellated RPE cell monolayer observed in a normal human eye (right), marked changes in RPE cell size and shape are observed in the human eye with geographic atrophy (left). These changes resemble those observed in eyes of mice wherein Dicer1 has been depleted in the RPE. RPE cell borders delineated by staining with anti-ZO-1 antibody (green) and nuclei stained by propidium iodide (red). Representative image shown. n=8. Scale bar, 50 μm.

FIG. 11 Conditional ablation of Drosha, Dger8, or Ago2 in the retinal pigmented epithelium (RPE) does not induce degeneration as seen in Dicer1-ablated mice. Fundus photographs (left column) show no significant degeneration following subretinal injection of AAV1-BEST1-Cre in mice “foxed” for Drosha, Dgcr8, or Ago2. Circular flash artifacts are seen near the centre of the fundus photographs. Injection site wound appears white in the fundus photograph of the Ago2/fox eye. RPE flat mounts (middle column) stained with anti-ZO-1 antibody (red) and Hoechst 33342 (blue) show normal tiling pattern of RPE with no gross disturbance of cell size or shape. RPE flat mounts (right column) stained with anti-Cre recombinase antibody (red) and Hoechst 33342 (blue) shows successful Cre expression in these mice eyes. Representative images shown. n=8-12. Scale bar (20 μm).

FIG. 12 Deficiency of Ago1, Ago3, Ago4, or Tarbp2 does not induce RPE degeneration. Mice deficient in Ago1 Ago3 Ago4, or Tarbp2 have normal retinal appearance on fundus photography (top row) and normal RPE monolayer architecture on ZO-1 stained (red) flat mounts (bottom row). Circular flash artifact is seen in the centre of the fundus photographs. Scale bar, 20 μm.

FIG. 13 Dicer1 mutant cells impaired in miRNA biogenesis do not have compromised cell viability. There was no difference in baseline cell viability between HCT-Dicer1−/− cells, which are impaired in miRNA biogenesis, and parent HCT116 cells over 3 days of analysis of cell proliferation. n=3. NS, not significant.

FIG. 14 Human geographic atrophy eye retinal pigmented epithelium contain Alu RNA sequences. a, Top: Typical Alu element with conserved structural regions (adapted from ref. 2). The left arm consists of RNA polymerase III binding sites (Box A and Box B). The right arm occasionally contains a terminal poly A tail that may be interspersed with non-A bases. The 5′ and 3′ regions of the Alu element are linked by the Pol II inhibitor a-amanitin. b, Alignment of Alu cDNA Sequences 1 and 2 isolated from human eyes with geographic atrophy to Alu Sq consensus sequence. These sequences contain the highly conserved 5′ Alu consensus elements (5′ characteristic Alu region—blue; RNA polymerase III promoter B box—red), with extensive heterogeneity located 3′ to the mid-sequence poly-A stretch that have been reported to exist in Alu sequences1−4. c, Northern blot shows that J2 anti-dsRNA antibody in an immuno-dot blot format. J2 antibody did not recognize rRNA or tRNA (negative controls), but did recognize RNA duplexes of 325-bp or 1-kbp in length (positive controls). b, Immunofluorescent imaging of human RPE cells transfected with pAlu shows that J2 recognizes Alu expressed in these cells (left panel). Specificity of staining confirmed by absence of staining with isotype control antibody (middle panel) and by the absence of immunodetection following transfection with pNull (right panel). Representative images shown. n=3. Scale bar (20 μm).

FIG. 15 J2 anti-dsRNA antibody recognizes Alu RNA. a, Alu RNA duplex isolated and cloned from the retinal pigmented epithelium (RPE) of a human eye with geographic atrophy was recognized by J2 anti-dsRNA antibody.
probe detects in vitro transcribed Alu RNA but not 7SL RNA in mouse liver (which lacks primate-specific Alu), and reprofiling these samples confirms specificity of the 7SL probe. d, DICER1 knockdown by antisense (as) oligonucleotide in human RPE cells does not, compared to control (Ctrl) as treatment, induce upregulation of several Pol II-transcribed genes (ADAR2, NICN, NLRP, SLFN 11) that contain embedded Alu sequences in their exons. n=3.

**FIG. 21** 7SL RNA is not regulated in geographic atrophy or by inhibition of DICER1 or Alu. a, 7SL RNA abundance was not different in the RPE of human eyes with geographic atrophy (GA) compared to the RPE of normal human eyes without GA (n=8). b, 7SL RNA abundance was not different in human RPE cells transfected with antisense oligonucleotide (as) targeting DICER1 from those transfected with control (Ctrl) as. n=3. c, 7SL RNA abundance was not different in human RPE cells transfected with antisense oligonucleotide (as) targeting Alu from those transfected with control (Ctrl) as. n=3. 7SL RNA abundance, relative to 18S RNA, was monitored by real-time RT-PCR. NS, not significant by Student t test.

**FIG. 22** Overexpression of B1 or B2 RNA induces RPE degeneration. Subretinal transfection of pB1 or pB2 RNAs, but not of pNull, induces RPE degeneration in wild-type mice. Top row shows fundus photographs demonstrating areas of degeneration outlined by blue arrowheads. Bottom row shows ZO-1 stained (red) RPE flat mounts demonstrated marked degeneration and disarray of the RPE cells in mice overexpressing B1 or B2 RNAs. Circular flash artifact is seen in the centre of the fundus photographs. n=4. Representative images shown. Scale bar, 20 μm.

**FIG. 23** Alu RNA enters retinal pigmented epithelium (RPE) cells in vivo. Subretinal administration of Alu RNA in wild-type mice achieved RPE cell delivery at 8 h after injection as monitored by real-time RT-PCR in isolated cell lysates (n=3).

**FIG. 24** Human GA Alu dsRNA does not induce RPE degeneration when cleaved by DICER1. a, Subretinal administration of a fully complementary synthetic Alu RNA (dsRNA) corresponding to the sequence of an Alu RNA isolated from a human eye with geographic atrophy (GA) induces RPE degeneration in wild-type mice. Vehicle administration does not damage the retina. Top panels show fundus photographs with the area of RPE degeneration outlined by blue arrowheads. Bottom panels show ZO-1 stained (red) RPE flat mounts demonstrated marked degeneration and disarray of the RPE cells in mice overexpressing Alu dsRNA (top). b, This Alu dsRNA did not induce RPE degeneration when it was first subjected to mock cleavage by recombinant DICER1. However, when subjected to mock cleavage by DICER1, this Alu dsRNA did induce RPE degeneration. n=4. Representative images shown. Scale bar, 20 μm.

**FIG. 25** RPE degeneration does not occur in response to a variety of structured RNAs. Subretinal transfection of transfer RNA (tRNA) or of plasmids coding for 7SL RNA, pri-miRNA-29b1 or pri-miRNA-26a2 in wild-type mice did not induce retinal toxicity that was evident on fundus photography. Circular flash artifact is seen in the centre of the fundus photographs. N=4. Representative images shown.

**FIG. 26** Alu RNA does not cause RPE degeneration via TLR3. a, Western blot shows that transfection of pAlu or pNull does not induce TLR3 phosphorylation, relative to the levels of the housekeeping protein Vinculin, in human RPE cells. b, Subretinal transfection of pAlu induced RPE degeneration in Tlr3−/− mice where pNull transfection did not do so. Representative images shown. n=4. Scale bar, 20 μm.

**FIG. 27** DICER1 reduction or Alu RNA augmentation induces caspase-3 activation. a, Immunolocalization of activated caspase-3 (red) in the RPE of human eyes with geographic atrophy (left panel). Specificity of immunolabeling revealed by absence of staining with isotype control antibody (middle panel) and in control eyes stained with antibody against cleaved caspase-3 (right panel). Autofluorescence of RPE and choroid seen in green channel. Nuclei stained by DAPI (blue). b, Flat mounts of BEST1 Cre; Dicer1−/− mice show evidence of caspase-3 activation (red staining, top left panel). Specificity of immunolabeling revealed by absence of staining with isotype control antibody (top right panel). No caspase-3 activation was detectable in the RPE of littermate control BEST1 Cre or Dicer1+/− mice (bottom panels). c, Human RPE cells transfected with Alu showed evidence of caspase-3 activation (red staining, top left panel). DAPI (blue staining) and merged images are also shown. Scale bars (20 μm, a; 10 μm, c). Representative images shown. n=4-6. d, Exposure of human RPE cells to Alu RNA induced dose-dependent increase in caspase-3 activation, as monitored by fluorometric plate assay. n=3, *P<0.05 compared to vehicle by Student t test. e, Transfection of human RPE cells with pAlu induced increase in caspase-3 activation. n=3, *P=0.47 by Student t test.

**FIG. 28** Alu RNA cleavage fragments do not modulate RPE degeneration. a, Transfection of pAlu induced cell death in human RPE cells. Cotransfection of DICER1-cleaved Alu RNA fragments did not change the degree of cell death. n=3. b, Subretinal transfection of DICER1-cleaved Alu RNA fragments (Frag) in wild-type mice did not cause RPE degeneration as seen by fundus photography (top left) or ZO-1-stained (red) RPE flat mounts (bottom left). Cotransfections of these fragments did not prevent the RPE degeneration induced by pAlu in wild-type mice (right panels). n=4. Representative images shown. Scale bar, 20 μm.

**FIG. 29** Impaired DICER1 processing of microRNAs does not increase Alu RNA abundance or modulate Alu RNA cytotoxicity. a, There was no significant difference (P>0.05) in Alu RNA transcript abundance between HCT116 parent cells and HCT mutant cells carrying a mutation in exon 5 (ex5) of DICER1 which renders it incapable of processing microRNAs. b, Transfection of anti-sense oligonucleotide (as) targeting DICER1 into HCT116 cells increased the abundance of Alu RNA transcripts compared to control anti-sense oligonucleotide (Ctrl) as (48 h). Transcript abundance monitored by real-time RT-PCR and normalized to 18S rRNA levels. c, Alu RNA induced similar levels of cell death in HCT116 parent and HCT-Dicer1−/− cells. *P<0.05 by Student t test. n=4-6.

**FIG. 30** Oxidative stress downregulates DICER1 in human RPE cells. Human retinal pigmented epithelium (RPE) cells exposed to varying concentrations of hydrogen peroxide (H2O2) display a dose- and time-dependent reduction in DICER1 mRNA abundance, as monitored by real-time RT-PCR and normalized to 18S rRNA levels. n=3.

**BRIEF DESCRIPTION OF THE SEQUENCE LISTING**

SEQ ID NO: 1 is an embodiment of a first strand of an siRNA provided in accordance with the presently-disclosed subject matter.
SEQ ID NO: 2 is an embodiment of a first strand of an siRNA provided in accordance with the presently-disclosed subject matter.

SEQ ID NO: 3 is an embodiment of a first strand of an siRNA provided in accordance with the presently-disclosed subject matter.

SEQ ID NO: 4 is an embodiment of a first strand of an siRNA provided in accordance with the presently-disclosed subject matter.

SEQ ID NO: 5 is an embodiment of a first strand of an siRNA provided in accordance with the presently-disclosed subject matter.

SEQ ID NO: 6 is an embodiment of a first strand of an siRNA provided in accordance with the presently-disclosed subject matter.

SEQ ID NO: 7 is a nucleotide sequence encoding a human DICER polypeptide, including all untranslated regions (GenBank Accession Number NM_177458).

SEQ ID NO: 8 is a cDNA sequence encoding a human DICER polypeptide.

SEQ ID NO: 9 is a polypeptide sequence for a human DICER polypeptide.

SEQ ID NO: 10 is a polypeptide sequence for a human DICER polypeptide, including residues 1276-1922 of SEQ ID NO: 9.

SEQ ID NO: 11 is a polypeptide sequence for a human DICER polypeptide, including residues 605-1922 of SEQ ID NO: 9.

SEQ ID NO: 12 is a polypeptide sequence for a human DICER polypeptide, including residues 1666-1922 of SEQ ID NO: 9.

SEQ ID NO: 13 is a polypeptide sequence for a human DICER polypeptide, including residues 1666-1912 of SEQ ID NO: 9.

SEQ ID NO: 14 is a polypeptide sequence for a human DICER polypeptide, including residues 1666-1786 and 1800-1912 of SEQ ID NO: 9.

SEQ ID NO: 15 is a polypeptide sequence for a human DICER polypeptide, including residues 1275-1824 of SEQ ID NO: 9.

SEQ ID NO: 16 is a polypeptide sequence for a human DICER polypeptide, including residues 1276-1824 of SEQ ID NO: 9.

SEQ ID NO: 17 is an mRNA sequence encoding a human DICER polypeptide.

SEQ ID NO: 18 is a polypeptide sequence for a Schizosaccharomyces pombe DICER polypeptide.

SEQ ID NO: 19 is an mRNA sequence encoding a Schizosaccharomyces pombe DICER polypeptide.

SEQ ID NO: 20 is a polypeptide sequence for a Giardia lamblia DICER polypeptide.

SEQ ID NO: 21 is an mRNA sequence encoding a Giardia lamblia DICER polypeptide.

SEQ ID NO: 22 is an embodiment of an antisense oligonucleotide sequence provided in accordance with the presently-disclosed subject matter.

SEQ ID NO: 23 is an embodiment of an antisense oligonucleotide sequence provided in accordance with the presently-disclosed subject matter.

SEQ ID NO: 24 is an embodiment of an antisense oligonucleotide sequence provided in accordance with the presently-disclosed subject matter.

SEQ ID NO: 25 is an embodiment of an antisense oligonucleotide sequence provided in accordance with the presently-disclosed subject matter.

SEQ ID NO: 26 is an embodiment of an antisense oligonucleotide sequence provided in accordance with the presently-disclosed subject matter.

DESCRIPTION OF EXEMPLARY EMBODIMENTS

The presently-disclosed subject matter includes methods for identifying Alu RNA inhibitors, and methods and compositions for inhibiting Alu RNA and therapeutic uses thereof.

As disclosed herein, Alu RNA (including Alu repeat RNA in human cells and B1 and B2, Alu-like element repeat RNAs) increases are associated with cells that are associated with certain conditions of interest. For example, Alu RNA increase is associated with the retinal pigment epithelium (RPE) cells of eyes with geographic atrophy. This increase of Alu RNA induces the death of RPE cells. Methods and compositions disclosed herein can protect a cell from Alu RNA-triggered cell death, thereby treating conditions associated with such cell death.

The presently-disclosed subject matter further includes methods useful for identifying an Alu RNA inhibitor and uses of such inhibitors, including therapeutic and protective uses. In some embodiments, the method makes use of a cultured cell wherein Alu RNA is upregulated. Candidate compounds can be screened using the cultured cell to determine efficacy as antagonists of Alu RNA. Candidate compounds include, for example, small molecules, biologics, and combinations thereof, such as compositions including multiple compounds. The term small molecules is inclusive of traditional pharmaceutical compounds. The term biologics is inclusive of polypeptides and nucleotides.

In some embodiments, the screening method includes providing a cell in culture wherein Alu RNA is upregulated; and contacting a candidate compound with the cell. The method can further include identifying a change in Alu RNA. For example, a measurable decrease in Alu RNA levels can be indicative of efficacy associated with the candidate compound. In some embodiments, wherein the increase in the Alu RNA is a measurable decrease in Alu RNA, the change is an indication that the candidate compound is an Alu RNA inhibitor. Such Alu RNA inhibitors can have utility for therapeutic applications as disclosed herein.

In some embodiments, the Alu RNA can be upregulated by decreasing native levels of DICER polypeptides in the cell using methods known to those skilled in the art. In some embodiments, the Alu RNA associated with cultured cell can be upregulated by using heat shock stress using methods known to those skilled in the art. In some embodiments, the cultured cell is an RPE cell.

Methods and compositions of the presently-disclosed subject matter for treating a condition of interest include inhibiting Alu RNA associated with a cell, such as a cell of a subject in need of treatment. Examples of conditions of interest include, but are not limited to: geographic atrophy, dry age-related macular degeneration, thalassemia, familial hypercholesterolemia, Dent’s disease, acute intermittent porphyria, anterior pituitary adenoma, Apert syndrome, Hemophilia A, Hemophilia B, glyceral kinase deficiency, autoimmune lymphoproliferative syndrome, X-linked agammaglobulinemia, X-linked severe combined immunodeficiency, adrenoleukodystrophy, Menkes disease, hyperimmunoglobulin M syndrome, retinal blinding, Type I anti-thrombin deficiency, Muckle-Wells syndrome, hypocalcemic hypercalcemia and hyperparathyroidism, cholinesterase deficiency, hereditary desmoid disease, chronic
hemolytic anemia, cystic fibrosis, broncho-oto-renal syndrome, lipoprotein lipase deficiency, CHARGE syndrome, Walker Warburg syndrome, Complement deficiency, Muco-1
lipidosis type II, Breast cancer, ovarian cancer, prostate cancer, von Hippel-Lindau disease, Hereditary non-polyposis colorectal cancer, multiple endocrine neoplasia type 1, hereditary diffuse gastric cancer, hepatoma, neurofibromatosis type 1, acute myeloid leukemia, T-acute lymphoblastic leukemia, and Ewing sarcoma.

As used herein, the terms treatment or treating relate to any treatment of a condition of interest, including but not limited to prophylactic treatment and therapeutic treatment. As such, the terms treatment or treating include, but are not limited to: preventing a condition of interest or the development of a condition of interest; inhibiting the progression of a condition of interest; arresting or preventing the development of a condition of interest; reducing the severity of a condition of interest; ameliorating or relieving symptoms associated with a condition of interest; and causing a regression of the condition of interest or one or more of the symptoms associated with the condition of interest.

As used herein, the term "subject" refers to a target of treatment. The subject of the herein disclosed methods can be a vertebrate, such as a mammal, a fish, a bird, a reptile, or an amphibian. Thus, the subject of the herein disclosed methods can be a human or non-human. Thus, veterinary therapeutic uses are provided in accordance with the presently disclosed subject matter.

In some embodiments, the condition of interest is geographic atrophy and the cell is an RPE cell. In this regard, a subject having age-related macular degeneration can be treated using methods and compositions as disclosed herein. As will be recognized by one skill in the art upon studying this application, inhibition of Alu RNA associated with a cell is achieved in a number of manners. For example, in some embodiments, inhibiting Alu RNA associated with a cell comprises increasing levels of a DICER polypeptide in the cell, for example, by overexpressing the DICER polypeptide in the cell. For another example, a DICER mRNA could be used. For another example, in some embodiments, inhibiting Alu RNA associated with a cell comprises administering an oligonucleotide or a small RNA molecule targeting the Alu RNA. As used herein, inhibiting Alu RNA associated with a cell refers to a reduction in the levels of Alu RNA inside and/or outside the cell in the extracellular space.

The term DICER Polypeptide refers to polypeptides known to those of ordinary skill in the art as DICER, including, but not limited to polypeptides comprising the sequences of SEQ ID NO: 9, 18, and 20, and functional fragments or functional variants thereof.

It is noted that one of ordinary skill in the art will be able to readily obtain publicly-available information related to DICER, including relevant nucleotide and polypeptide sequences included in publicly-accessible databases, such as GENBANK®. Some of the sequences disclosed herein are cross-referenced to GENBANK® accession numbers, e.g., GenBank Accession Number NM_177438. The sequences cross-referenced in the GENBANK® database are expressly incorporated by reference as are equivalent and related sequences present in GENBANK® or other public databases. Also expressly incorporated herein by reference are all annotations present in the GENBANK® database associated with the sequences disclosed herein. Unless otherwise indicated or apparent, the references to the GENBANK® database are references to the most recent version of the database as of the filing date of this application.

The terms "polypeptide", "protein", and "peptide", which are used interchangeably herein, refer to a polymer of the 20 protein amino acids, or amino acid analogs, regardless of its size. The terms "polypeptide fragment" or "fragment", when used in reference to a reference polypeptide, refers to a polypeptide in which amino acid residues are deleted as compared to the reference polypeptide itself, but where the remaining amino acid sequence is usually identical to the corresponding positions in the reference polypeptide. Such deletions can occur at the amino-terminus (e.g., removing residues 1-604, 1-1274, 1-1275, or 1-1665 of SEQ ID NO: 9) or carboxy-terminus of the reference polypeptide (e.g., removing residues 1825-1922, or 1913-1922 of SEQ ID NO: 9), from internal portions of the reference polypeptide (e.g., removing residues 1787-1799 of SEQ ID NO: 9), or a combination thereof.

A fragment can also be a "functional fragment," in which case the fragment retains some or all of the activity of the reference polypeptide as described herein. For example, in some embodiments, a functional fragment of the polypeptide of SEQ ID NO: 9 can retain some or all of the ability of the polypeptide of SEQ ID NO: 9 to degrade Alu RNA. Examples of functional fragments of the polypeptide of SEQ ID NO: 9 include the polypeptides of SEQ ID NOS: 10-16. Additional examples include, but are not limited to, the polypeptide of SEQ ID NO: 9, including the following residues: 605-1922, 605-1912, 1666-1912, 605-1786 and 1800-1922, 605-1786 and 1800-1912, 1666-1786 and 1800-1912, 1276-1922, 1276-1912, 1276-1786 and 1800-1922, 1276-1786 and 1800-1912, 1275-1824, or 1276-1824.

The terms "modified amino acid", "modified polypeptide", and "variant" refer to an amino acid sequence that is different from the reference polypeptide by one or more amino acids, e.g., one or more amino acid substitutions. A variant of a reference polypeptide also refers to a variant of a fragment of the reference polypeptide, for example, a fragment wherein one or more amino acid substitutions have been made relative to the reference polypeptide. A variant can also be a "functional variant," in which the variant retains some or all of the activity of the reference protein as described herein. The term functional variant includes a functional variant of a functional fragment of a reference polypeptide.

In some embodiments, the DICER Polypeptide can be overexpressed in the cell using a vector comprising a nucleotide encoding the DICER polypeptide, for example, the nucleotide of SEQ ID NO: 9, or an appropriate fragment thereof, or a nucleotide encoding a DICER Polypeptide, for example, a nucleotide encoding SEQ ID NOS: 9, 10, 11, 12, 13, 14, 15, 16, 18, or 20. As will be recognized by those skilled in the art, the vector can be a plasmid vector or a viral vector (e.g., adeno-associated virus, lentivirus, adenovirus).

As noted above, in some embodiments, inhibiting Alu RNA comprises use of a DICER mRNA. In some embodiments, a functional fragment of a DICER mRNA could be used. In some embodiments, a DICER mRNA having the sequence of SEQ ID NOS: 17, 19, or 21, or a functional fragment thereof could be used. In some embodiments an mRNA encoding a DICER Polypeptide could be used, for example, an mRNA encoding SEQ ID NOS: 9, 10, 11, 12, 13, 14, 15, 16, 18, or 20.

As noted above, in some embodiments, inhibiting Alu RNA comprises administering an oligonucleotide or a small RNA molecule targeting the Alu RNA. Such nucleotides can target and degrade Alu RNA.
As such, in some embodiments, a method is provided including administering an oligonucleotide targeting Alu RNA. Examples of oligonucleotides targeting Alu RNA include those set forth in SEQ ID NOs: 22-26. In some embodiments, more than one oligonucleotide is administered.

In some embodiments, a method is provided including administering an siRNA targeting Alu RNA. Examples of siRNAs for targeting Alu RNA include those set forth in SEQ ID NOs: 1-6.

The details of one or more embodiments of the presently-disclosed subject matter are set forth in this document. Modifications to embodiments described in this document, and other embodiments, will be evident to those of ordinary skill in the art after a study of the information provided in this document. The information provided in this document, and in particular, the specific details of the described exemplary embodiments, is provided primarily for clearness of understanding and no unnecessary limitations are to be understood therefrom. In case of conflict, the specification of this document, including definitions, will control.

While the terms used herein are believed to be well understood by one of ordinary skill in the art, definitions are set forth to facilitate explanation of the presently-disclosed subject matter.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the presently-disclosed subject matter belongs. Although any methods, devices, and materials similar or equivalent to those described herein can be used in the practice or testing of the presently-disclosed subject matter, representative methods, devices, and materials are now described.

Following long-standing patent law convention, the terms "a", "an", and "the" refer to "one or more" when used in this application, including the claims. Thus, for example, reference to "a cell" includes a plurality of such cells, and so forth.

Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as reaction conditions, numerical parameters set forth in this specification and other data included in the present application, including the claims, are to be understood as being modified in all instances by the term "about", i.e., ±1%, ±2%, ±5%, ±10%, and ±15%, when used in this application, including the claims.

The following examples may include compilations of data that are representative of data gathered at various times during the course of development and experimentation related to the present invention.

**EXAMPLES**

DICER1 Reduction in Geographic Atrophy

In human donor eyes with geographic atrophy (n=10), the present inventors found using real-time RT-PCR that DICER1 mRNA abundance was reduced in the macular RPE by 65±3% (mean±SEM; P=0.0036; Mann-Whitney U test) compared to age-similar human eyes without geographic atrophy (n=11) (FIG. 1a). Because the best understood function of DICER1 is miRNA generation5, the present inventors measured the expression of other enzymes involved in miRNA biogenesis. The abundance of the genes encoding DROSHA or the double stranded RNA (dsRNA) binding protein DGC8, which form a complex that processes pri-miRNAs into pre-miRNAs6, was not reduced in the RPE of human eyes with geographic atrophy. There was also no reduction in the expression of the gene encoding Argonaute 2 (AGO2, encoded by EIF2C2), the core component of the miRNA effector complex7,8, in the RPE of human eyes with geographic atrophy. Corroborating the mRNA data, the present inventors observed a marked reduction of DICER1 protein expression in the RPE layer of human eyes with geographic atrophy compared to controls in Western blot (FIG. 1b and FIG. 6) and immunohistochemistry analyses (FIG. 1c). Interestingly, DICER1 mRNA and protein abundance in the adjacent neural retina was similar between the two groups (FIG. 7).

Because DICER1 downregulation is observed in some cell types in culture conditions in response to various chemical stresses9, the present inventors wondered whether DICER1 reduction in geographic atrophy might be a common downstream pathway in dying retina. DICER1 protein levels were not reduced in the RPE of human eyes with several other retinal disorders such as vitelliform macular dystrophy, retinitis pigmentosa, or retinal detachment (FIG. 8). Also, Dicer1 mRNA abundance in the RPE in two animal models of retinal degeneration—Ce2−/−Cer2−/− (refs. 9,10) and C57/6 Heph−/− mice9,11—was unchanged compared to their background strains (FIG. 8). Gene expression studies in numerous other mouse models of retinal degeneration also have not reported modulation of Dicer1 (Supplemental Notes). These data argue that DICER1 depletion in the RPE of eyes with geographic atrophy is not a generic response of damaged or dying retinal cells in vivo.

DICER1 Depletion Induces RPE Degeneration

To determine the functional consequence of reduced DICER1 levels, the present inventors conditionally ablated Dicer1 in mouse RPE cells by interbreeding "foxed" Dicer1 mice12 (Dicer112) with BEST1 Cre mice13, which express Cre recombinase under the control of the RPE cell-specific BEST1 promoter. BEST1 Cre; Dicer112 mice uniformly exhibited dramatic RPE cell degeneration (FIG. 1d-f) that was evident by the time of weaning. None of the littermate controls exhibited similar pathology. The present inventors also deleted Dicer1 in adult mouse RPE by subretinal injection of an adenovirus-associated viral vector coding for Cre recombinase under the control of the BEST1 promoter14 (AAV1-BEST1-Cre) in Dicer112 mice (FIG. 9). These eyes uniformly displayed RPE cell degeneration at 28 days after injection similar to that observed in mice depleted of Dicer1 expression during development (FIG. 1g-i; FIG. 9). In
contrast, neither the contralateral eyes of Dicer1
mice that underwent subretinal injection of AAV1-BEST1-GFPnor
the eyes of wild-type mice injected with subretinal AAV1-
BEST1-Cre developed RPE cell degeneration (Fig. 1g-i and
Fig. 9). The RPE cell morphogenesis in mice depleted of
Dicer1 expression resembled that observed in the eyes of
humans with RPE atrophy due to AMD (Fig. 10). When
cultured RPE cells isolated from Dicer1 mice were
infected with an adenoviral vector coding for Cre recombi-

nase (Ad-Cre), the present inventors observed a reduction of
cell viability compared to infection with Ad-Null (Fig. 1j).
Similarly, antisense oligonucleotide mediated knockdown of
DICER1 in human RPE cells resulted in increasing cell
death over time (Fig. 1k). Collectively, these data support the
hypothesis that DICER1 dysregulation is involved in the
pathogenesis of geographic atrophy.

DICER1 Depletion Phenotype not Due to miRNA Dys-
regulation

The present inventors tested whether depletion of other
enzymes involved in miRNA biogenesis also would induce
RPE degeneration. Subretinal injection of AAV1-BEST1-
Cre in Drosophila (ref. 13), Dger8^- (refs. 15,16), or bg20
mice17 did not result in the dramatic RPE cell damage that
was evident in similarly treated Dicer2^- mice (Fig. 11).
These data suggest that miRNA imbalances are not respon-
sible for RPE degeneration induced by DICER1 depletion.
However, the present inventors and others have reported 18,19
that a small subset (approximately 7%) of mammalian
miRNAs is generated by Dicer1 independent of Drosha and
Dgcr8. There is also debate as to whether Ago2 is essential
for miRNA function: Ago2 deficiency leads to global reduc-
tion of miRNA expression uncompensated by other three
Ago proteins in mice20-21 and in mouse embryonic fibro-

blasts and oocytes22-23, yet functional redundancy among
Argonaute proteins has been reported in mouse embryonic
stem cells24. The present inventors found no RPE degener-

ation in mice deficient in Ago1, Ago3, or Ago4 (Fig. 12).
TRBP (the human immunodeficiency virus transactivating
response RNA-binding protein encoded by Tarbp2) recruits
DICER1 to the four Argonate proteins to enable miRNA
processing and RNA silencing (ref 24 and R. Shiekhattar,
personal communication); Tarbp2^- mice too had no RPE
degeneration (Fig. 12). These data suggest that RPE degen-

eration induced by Dicer1 ablation involves a mechanism
specific to Dicer1 and not to miRNA machinery in general.

To further investigate whether miRNA imbalances might
contribute to the phenotype observed in the setting of
dicer1 depletion, the present inventors studied human
HCT116 colon cancer cells in which the helicase domain in
exon 5 of DICER1 was disrupted. Despite the impairment of
miRNA biogenesis in these HCT-DICER1^- cells, there
was no difference between HCT-DICER1^- and parent
HCT116 cells in baseline cell viability (Fig. 13). Collect-

ively, these findings suggest that the principal biological
effect of DICER1 deficit contributing to the development of
geographic atrophy is not miRNA dysregulation. The find-

ings do not, however, exclude the possibility that miRNA
dysregulation could promote geographic atrophy through
other pathways.

Alu RNA Accumulation in Geographic Atrophy

Because miRNA perturbations could not be implicated,
the present inventors speculated that impaired processing of
other dsRNAs might be involved. Using an antibody25,26 that
recognizes long dsRNA (J2), the present inventors
detected abundant dsRNA immunoactivity in the macular
RPE of human eyes with geographic atrophy (n=10; Fig.
2a-c). In contrast, no J2 immunoreactivity was observed in
eyes without geographic atrophy (n=10; Fig. 2d). To iden-

ify this dsRNA species, the present inventors immunopre-

cipitated RPE lysates with J2 antibody and then sequenced
the dsRNA using a 14 RNA ligase-aided, adaptor-based
PCR amplification strategy. Interestingly, approximately
300-nt long dsRNA species were found in the macular RPE
of human eyes with geographic atrophy (12/12) but not in
eyes without geographic atrophy (0/18) (P=1.2x10^-8 by
Fisher’s exact test) (Fig. 2e).

The present inventors recovered clones from 8 of the 12
geographic atrophy eyes and identified two distinct
sequences with high homology (E=3.3x10^-103; 1.1x10^-76)
to Alu repeat RNAs (Fig. 14). These sequences showed
homology to the Alu Sq subfamily consensus sequence.
Apart from mitochondrial RNAs that were occasionally
found in the RPE of both geographic atrophy and normal
eyes, Alu RNAs were the only repetitive RNAs identified
specifically in the geographic atrophy samples. The present
inventors confirmed that the J2 monoclonal antibody recog-
nized Alu RNA both in immunoblotting and in immuno-

fluorescence assays (Fig. 15). The present inventors also
detected a greater than 40-fold increase in the abundance of
Alu RNAs in the RPE of human eyes with geographic
atrophy compared to control eyes (n=7), but no significant
difference in Alu RNA abundance was detected in the
adjacent neural retina between the two groups (Fig. 2f,
Fig. 16). The present inventors did not identify exact matches
to these Alu sequences in the reference human genome. This
could be attributed to genetic variations or regions not
represented in the reference genome or to chimeric Alu
formation. Further studies are needed to elucidate the
genomic origin of and regulatory factors involved in tran-
scription of these Alu RNAs.

DICER1 Depletion Induces Alu RNA Accumulation

The present inventors tested whether Alu RNA accumu-
lation in the RPE of geographic atrophy was the result of
deficient DICER1 processing activity. DICER1 knockdown
in human RPE cells using antisense oligonucleotides
resulted in increasing Alu RNA accumulation over time
(Fig. 3a, Fig. 17). Similarly, Ad-Cre infection of RPE cells
isolated from Dicer1^- mice resulted in accumulation of B1
and B2 repeat RNAs (Fig. 3b, c), which are Alu-like short
interspersed repetitive elements in the mouse. Interestingly,
DICER1 was expressed in both the nucleus and cytoplasm
of RPE cells and its depletion led to accumulation of
Alu/B1/B2 RNA in both cellular compartments (Fig. 3d-
Fig. 18). In addition, recombinant DICER1 degraded Alu
RNA, and the biochemical specificity of this cleavage was
confirmed by the inability of heat-denatured DICER1 to
degradate Alu RNA (Fig. 3e, f). Alu RNA was detected in
human RPE cells reduced Alu RNA abundance following enforced expression of Alu RNA (Fig. 3f), consistent with degradation of these repetitive transcripts by
DICER1 in vivo. Collectively these data confirm that
DICER1 dysregulation can trigger Alu/B1/B2 RNA accu-
mulation.

Because cell stresses such as heat shock or viral infection
can induce generalized retrotransponson activation, the pres-
ent inventors wondered whether Alu RNA accumulation in
geographic atrophy might be a generic response in dying
retina. However, in the RPE of human eyes with geographic
atropathy and in DICER1-depleted human RPE cells, there
was no increase in the abundance of RNAs coded by L1.3
(a long interspersed repetitive element), human endogenous
retrovirus-W envelope (a long terminal repeat retrotranspo-
sion), or L1Y3 (a repetitive small cytoplasmic Ro RNA) (Fig.
19. These data demonstrate that Alu RNA accumulation is a biologically specific response to DICER1 depletion.

To determine whether Alu RNA accumulation was derived from RNA polymerase II (Pol II) or Pol III transcriptions, the present inventors performed experiments using α-amanitin (a Pol II inhibitor) and tagetitoxin (a Pol III inhibitor). Alu RNA upregulation induced by DICER1 knockdown was inhibited by tagetitoxin but not α-amanitin (FIG. 20). The present inventors also found using Northern blotting that Alu RNA from the RPE of human eyes with geographic atrophy was approximately 300 nucleotides in length, consistent with the length of non-embedded Pol III Alu transcripts. Because homology between Alu RNA and 7SL RNA, the evolutionary precursor of Alu, can complicate interpretation of northern blot analysis, the present inventors reprobed these samples using a probe that specifically detects the non-Alu "S domain" of 7SL RNA. In contrast to the increased amounts of RNA species detected by the Alu-targeting probe in geographic atrophy RPE, there was no difference in 7SL RNA abundance. The present inventors also confirmed that the Alu probe did not detect endogenous 7SL RNA under the stringent conditions the present inventors employed. Corroborating these data, real-time RT-PCR analysis showed that 7SL RNA was not dysregulated in the RPE of human eyes with geographic atrophy or in DICER1-depleted human RPE cells (FIG. 21).

DICER1 knockdown also did not induce upregulation of several Pol II-transcribed genes (ADAR2, NICN, NLRP, SLFN 11) that contain embedded Alu sequences in their exons. Collectively, these data suggest that Alu RNA detected in the RPE of human eyes with geographic atrophy are primary Alu transcripts and not passenger or bystander sequences embedded in other RNAs. Conclusive assignment of these Alu sequences as Pol III transcripts must await precise determination of their transcription start site.

Alu RNA Induces RPE Degeneration

Next the present inventors tested whether accumulation of Alu RNA might promote the development of geographic atrophy. Transfecting human or wild-type mouse RPE cells with a plasmid coding for Alu (pA!u) reduced cell viability (FIG. 4a). Subretinal transfection of plasmids coding for two different Alu RNAs or for B1 or B2 RNAs induced RPE degeneration in wild-type mice (FIG. 4b, FIG. 22, and data not shown). Treatment of human RPE cells with a recombinant 281 nucleotide (nt)-long Alu RNA that is identical to a Pol III derived Alu RNA isolated from a human embryonal carcinoma cell line, i.e., a single RNA strand that folds into a defined secondary structure, resulted in a dose-dependent increase in cell death (FIG. 4c). These findings suggest that endogenous DICER1 can degrade small amounts of Alu RNA but are overwhelmed by high levels. Consistent with this concept, overexpression of DICER1 blocked pAlu-induced cell death in human RPE cells (FIG. 4d) and RPE degeneration in wild-type mice (FIG. 4e).

The present inventors verified that subretinal injection of Alu RNA resulted in its delivery to RPE cells in wild-type mice (FIG. 23), consistent with the ability of long RNAs with duplex motifs to enter cells28. The present inventors then cloned a 302-nt long Alu RNA isolated from the RPE of a human eye with geographic atrophy and transcribed it in vitro to generate partially and completely annealed structures that mimic Alu RNAs transcribed by Pol III and Pol II, respectively. Subretinal injection of either of these Alu RNAs resulted in RPE degeneration in wild-type mice (FIG. 4f, FIG. 24), supporting the assignment of disease causality in accord with the molecular Koch’s postulates. In contrast, subretinal injection of these Alu RNAs digested with DICER1 did not induce RPE degeneration in wild-type mice (FIG. 4g, FIG. 24). When these Alu RNAs were subjected to mock DICER1 digestion, they retained their ability to induce RPE degeneration, suggesting a role for DICER1 in protecting against Alu RNA-induced degeneration.

The present inventors tested whether other structured RNAs of similar length as Alu would damage the retina. Subretinal transfection of transfer RNA or plasmids coding for 7SL RNA or two different primary miRNAs did not induce RPE degeneration in wild-type mice (FIG. 25). The present inventors reported that chemically synthesized dsRNAs that mimic viral dsRNA can induce RPE degeneration by activating toll like receptor-3 (TLR3)29, a pattern receptor that generically recognizes dsRNA. However, transfection of a plasmid coding for Alu RNA did not induce TLR3 phosphorylation in human RPE cells and did induce RPE degeneration in Tlr3−/− mice (FIG. 26). These results indicate that the ability of Alu RNA to induce RPE degeneration cannot be attributed solely to its repetitive or double stranded nature, as it exerted effects distinct from other structured dsRNAs of similar length.

The mechanism of RPE cell death in geographic atrophy has not been previously defined. DNA fragmentation has been identified in RPE cells in human eyes with geographic atrophy30, and DICER1 knockdown has been associated with induction of apoptosis in diverse tissues12,31. The present inventors now provide evidence of caspase-3 cleavage in regions of RPE degeneration in human eyes with geographic atrophy (FIG. 27). Caspase-3 cleavage was also observed in the RPE cells of BEST1Cre; Dicer1−/− mice and in Alu RNA-stimulated or -overexpressing human RPE cells. These data suggest a role for Alu RNA-induced RPE cell apoptosis triggered by DICER1 dysregulation in geographic atrophy.

Although the present inventors show that Alu RNA induces RPE degeneration, the presented observations could be consistent with the idea that an imbalance in small RNA species produced from long Alu RNAs could contribute to the RPE degeneration phenotype. To study this question, the present inventors exposed human RPE cells or wild-type mice to DICER1 cleavage fragments of Alu RNA. Subretinal transfection of these fragments alone in wild-type mice had no detectable effect on RPE cell morphology, and co-administering these fragments did not prevent RPE cell degeneration induced by subretinal transfection of a plasmid coding for Alu RNA (FIG. 28). Similarly, these fragments did not prevent human RPE cell death induced by overexpression of Alu RNA. These data suggest that upregulation of long Alu RNA rather than imbalance in Alu RNA-derived small RNA fragments is responsible for RPE degeneration induced by DICER1 reduction.

As these experiments were performed with in vitro cleavage fragments the present inventors cannot be certain whether in vivo cleavage fragments would function similarly. However, Alu RNAs with varying sequences induced RPE degeneration in vivo. Because the cleavage fragments of these different Alu RNAs would not be identical it is unlikely that they all execute identical biological functions, particularly if they functioned as miRNAs. Another line of evidence that Alu RNA, and not its cleavage fragments, is responsible for RPE degeneration comes from functional rescue experiments (see below) wherein antisense-mediated inhibition of Alu RNA blocks human RPE cell death induced by DICER1 knockdown and inhibition of B1/B2 RNA blocks RPE degeneration in Dicer1-depleted mice and mouse RPE cells. Because these antisense treatments would not be expected to alter the reduced levels of DICER1-
cleaved Alu/B1/B2 RNA fragments, the imbalance in these fragments is unlikely to have induced RPE degeneration. Nevertheless, subtle functions of these small RNAs in modulating Alu RNA induced pathology cannot be excluded.

To dissect the contribution of Alu RNA accumulation versus that of miRNA dysregulation to RPE degeneration in the context of reduced DICER1 expression, the present inventors re-examined HCT-DICER1<sup>flf</sup> cells in which miRNA biogenesis is impaired but long dsRNA cleavage is preserved due to the intact Rnase III domains. The present inventors found no significant difference in Alu RNA levels between HCT-DICER1<sup>flf</sup> and parent HCT116 cells (FIG. 29). In contrast, when DICER1 was knocked down by antisense oligonucleotides in HCT116 cells, increased Alu RNA accumulation was observed. Also, Alu RNA induces similar levels of cytotoxicity in HCT-DICER1<sup>flf</sup> and parent HCT116 cells, suggesting that coexisting miRNA expression deficits do not augment Alu RNA induced RPE degeneration. In conjunction with the discordance in the RPE degeneration phenotype between ablation of DICER1 and that of various other small RNA biogenesis pathway genes in mice, the findings suggest that Alu RNA accumulation is critical to cytotoxicity induced by DICER1 reduction.

RPE Degeneration Blocked by Alu RNA Inhibition

The present inventors then tested whether the cytotoxic effects of DICER1 reduction could be attributed to Alu RNA accumulation. DICER1 Knockdown in human RPE cells by antisense oligonucleotides reduced cell viability (FIG. 5a). This cytotoxic effect of DICER1 reduction was inhibited by antisense oligonucleotides targeting Alu RNA sequences but not by a scrambled antisense control (FIG. 5a, b and FIG. 21). Ad-Cre infection of RPE cells isolated from Dicer1<sup>−/−</sup> mice resulted in reduced cell viability, and this was blocked by antisense oligonucleotides targeting both B1 and B2 repeat RNAs but not by a scrambled antisense control (FIG. 5a, c, d). Subretinal administration of antisense oligonucleotides that reduced accumulation of B1 and B2 RNAs also inhibited RPE degeneration in Dicer1<sup>−/−</sup> mice treated with AAV1-BEST1-Cre (FIG. 5e, f), providing evidence of in vivo functional rescue.

The present inventors tested whether Alu inhibition also rescued miRNA expression deficits as a potential explanation for the functional rescue of RPE degeneration induced by DICER1 depletion. As expected, DICER1 Knockdown in human RPE cells reduced the abundance of numerous miRNAs including let-7a, which is ubiquitously expressed, miR-184, miR-204/211, and miR-221/222, which are enriched in the RPE, and miR-320a, and miR-484 and miR-877, which are DROSHA/DGCR8-independent and DICER1-dependent (FIG. 5g). However, inhibition of Alu RNA did not lead to recovery of miRNA expression in these DICER1-depleted cells. Thus the rescue of RPE cell viability by Alu RNA inhibition despite the persistence of global miRNA expression deficits argues that RPE degeneration induced by DICER1 deficit is due to Alu RNA accumulation and not miRNA dysregulation.

These data, taken together, support a model in which primary Alu transcripts are responsible for the observed RPE degeneration. Whether similar pathology can also result from upregulation of as yet undefined Pol II transcripts with embedded Alu sequences is an intriguing possibility that may be addressed in future studies. Importantly, the present inventors show here that primary Alu transcripts are elevated in human disease, that Alu transcripts recapitulate disease in relevant experimental models, and that targeted suppression of Alu transcripts successfully inhibits this pathology. These observations have direct relevance for clinical strategies to prevent and treat geographic atrophy.

Discussion

The findings elucidate a critical cell survival function for DICER1 by functional silencing of toxic Alu transcripts. This unexpected function suggests that RNAi-independent mechanisms should be considered in interpreting the phenotypes of systems in which DICER1 is dysregulated. For example, it would be interesting to test the speculation that DICER1 ablation induced cell death in mouse neural retina<sup>32</sup> and heart<sup>33</sup> might also involve B1/B2 RNA accumulation. More broadly, recognition of DICER1’s hitherto unidentified function as an important controller of transcripts derived from the most abundant repetitive elements in the human and mouse genomes can illuminate new functions for RNases in cytoprotective surveillance. DICER1 expression is reduced in geographic atrophy and partial loss of DICER1 promotes RPE degeneration; thus the present inventors could speculate that loss of heterozygosity in DICER1 may underlie the development of geographic atrophy, similar to its function as a haploinsufficient tumor suppressor<sup>34</sup>-<sup>36</sup>. This also is, to our knowledge, the first example of how Alu could cause a human disease via direct RNA cytotoxicity rather than by inducing chromosomal DNA rearrangements or insertional mutagenesis through retrotransposition, which have been implicated in diseases such as α-thalassemia<sup>37</sup>, colon cancer<sup>38</sup>, hypercholesterolemia<sup>39</sup>-<sup>40</sup>, and neurofibromatosis<sup>41</sup>. Future studies can be employed to determine the precise chromosomal locus of the Alu RNA elements that accumulate in geographic atrophy and the nature of transcriptional and post-transcriptional machinery that enable their biogenesis.

In addition to processing miRNAs<sup>3</sup>, DICER1 has been implicated in heterochromatin assembly<sup>42</sup>-<sup>43</sup>. Since Alu repeat elements are abundant within heterochromatin<sup>44</sup>, it would be interesting to investigate whether perturbations in centromeric silencing also underlie the pathogenesis of geographic atrophy. Indeed, the finding that chromatin remodelling at Alu repeats can regulate miRNA expression<sup>45</sup> raises the intriguing possibility of other types of regulatory interactions between DICER1 and Alu. It also remains to be investigated whether centromeric satellite repeats that have been described to accumulate in Dicer1-null mouse embryonic stem cells<sup>46</sup>-<sup>47</sup> might be involved in the pathogenesis of geographic atrophy.

In the mouse germline, Dicer1 has been implicated in the generation of endogenous small interfering RNAs (endo-siRNAs) from repeat elements<sup>8</sup>-<sup>9</sup>. If this process is conserved in mammalian somatic tissues, it would be interesting to learn whether endo-siRNAs serve a homeostatic function in preventing the development of geographic atrophy. A recent study in nematodes demonstrated that cispases can cleave Dicer1 and convert it into a DNase that promotes apoptosis<sup>50</sup>. The finding that Alu RNA can induce caspase activation therefore introduces the possibility of bidirectional regulation between DICER1 and Alu that could trigger feed-forward loops that further amplify the disease state.

The inciting events that trigger an RPE-specific reduction of DICER1 in patients with geographic atrophy remain to be determined. Potential culprit could include oxidative stress, which is postulated to underlie AMD pathogenesis<sup>2</sup>, as the present inventors found that exposure to hydrogen peroxide downregulates DICER1 in human RPE cells (FIG. 30). While the upstream triggers of DICER1 dysregulation and the possible role of other DICER-dependent, DROSHA/ DGCR8-independent small RNAs in geographic atrophy await clarification, the ability of Alu RNA antisense oligonucleotides to inhibit RPE degeneration in mouse disease models suggests that these Alu-derived toxic small RNAs are a key target for therapy development.
nucleotides to inhibit RPE cell death induced by DICER1 depletion provides a rationale to investigate Alu RNA inhibition or DICER1 augmentation as potential therapies for geographic atrophy.

**Additional Notes**

DICER1 mRNA levels are not modulated in multiple mouse models of retinal degeneration including light damage, hyperoxia, retinal detachment, Cx3CR1−/− mice, Rslh1−/− mice, rd1 mice, cpfl1 mice, or Mitf mice. DICER1 abundance also is not reduced in mice models of cellular stress in the retina including exposure to advanced glycation endproducts or retinal detachment. Therefore, DICER1 downregulation is not a generic late-stage stress response in the retina.

**Materials and Methods**

**Animals**

All animal experiments were approved by institutional review committees and the Association for Research in Vision and Ophthalmology. C57Bl/6J and Dicer1−/− mice were purchased from The Jackson Laboratory. Transgenic mice that express Cre recombinase in the retinal pigmented epithelium under the control of the human bestrophin-1 promoter (BEST1 Cre mice), DGCGR89F, DrosulaF, Tarbp2−/−, Ccl2−/− Ccr2−/−, and Cpx−/− Hepha−/− mice have been previously described and mice deficient in Ago1, Ago3, or Ago4 (ref. 73) are generously provided by A. Tarakhovsky. For all procedures, anesthesia was achieved by intraperitoneal injection of 50 mg/kg ketamine hydrochloride (Pf. Dodge Animal Health) and 10 mg/kg xylazine (Phoenix Scientific), and pupils were dilated with topical 1% tropicamide (Alcon Laboratories).

**Fundus Photography.**

Retinal photographs of dilated mouse eyes were taken with a TCR-50 IX camera (Topcon) linked to a digital imaging system (Sony).

**Human Tissue.**

Donor eyes or ocular tissues from patients with geographic atrophy due to AMD or patients without AMD were obtained from various eye banks in Australia and the United States of America. These diagnoses were confirmed by dilated ophthalmic examination prior to acquisition of the tissues or eyes or upon examination of the eye globes post mortem. The study followed the guidelines of the Declaration of Helsinki institutional review boards granted approval for allocation and histological analysis of specimens.

**Immunolabeling.**

Human eyes fixed in 2-4% paraformaldehyde were prepared as eyecups, cryoprotected in 10 μm sections. Depigmentation was achieved using 0.25% potassium permanganate and 0.5% oxalic acid. Immunohistochemical staining was performed with the mouse antibody against dsRNA (1:1,000, clone J2, English & Scientific Consulting) or rabbit antibody against human DICER1 (1:100, Santa Cruz Biotechnology). Isotype IgG was substituted for the primary antibody to assess the specificity of the staining. Bound antibody was detected with biotin-conjugated secondary antibodies (Vector Laboratories). Slides were further incubated in alkaline phosphatase-streptavidin solution (In-vitrogen) and the enzyme complex was visualized by Vector Blue (Vector Laboratories). Levamisole (Vector Laboratories) was used to block endogenous alkaline phosphatase activity. Slides were washed in PBS, rinsed with deionized water, air-dried, and then mounted in Clear Mount (EMS). Mouse RPE/choroid flat mounts were fixed with 4% paraformaldehyde or 100% methanol and stained with rabbit antibodies against human zonula occludens-1 (1:100, Invitrogen), Cre recombinase (1:1000, EMD4Biosciences), or human cleaved caspase-3 (1:200, Cell Signaling) and visualized with Alexa594- or Cy5-conjugated secondary antibodies. Both antibodies are cross-reactive against the mouse homologues. Primary human RPE cells were grown to 70-80% confluency in chamber slides (Lab-Tek). After 24 h of transfection with pAlu or pUC19, cells were fixed in acetone for 10 min at −20° C. Cells were blocked with PBS-3% BSA and incubated with mouse antibody against dsRNA (1:500, clone J2) overnight at 4° C. and visualized with Alexa Fluor 488-conjugated secondary antibodies. For DICER1 staining, cells were fixed in methanol/acetone (7:3) for 30 min on ice, blocked with PBS-3% BSA-5% FBS, incubated with rabbit antibody against human DICER1 (1:100, Santa Cruz Biotechnology) overnight at 4° C., and visualized with goat-antirabbit-conjugated-594 or Cy5-conjugated secondary antibodies. After DAPI counterstaining, slides were cover slipped in Vectorshield (Vector Laboratories). Images were obtained using the Leica SP-5 or Zeiss Axio Observer Z1 microscopes.

**Histology.**

Mouse eyes were fixed with 4% paraformaldehyde and 3.5% glutaraldehyde, postfixed in 2% osmium tetroxide, and dehydrated in ethanol and embedded. Semi-thin (1 μm) sections were cut and stained with toluidine blue. Bright field images were obtained using the Zeiss Axio Observer Z1 microscope.

**Subretinal Injection.**

Subretinal injections (1 μL) in mice were performed using a Pico-Injector (PLI-100, Harvard Apparatus). In vivo transfection of plasmids coding for DICER1 (ref. 74), Alu Ya5 (ref. 75), Alu Yb9 (ref. 76), 7SL RNA (ref. 77), pri-miR20b1 (Addgene), or pri-miR20a2 (Addgene) and bovine tRNA (Sigma-Aldrich) (0.5 mg/mL) was achieved using 10% Neuroporter (Genlantis). AAV1-BEST1-Cre or AAV1-BEST1-GFP were injected at 1.0x1011 f.u./ml and recombinant Alu RNAs (1: a single RNA strand of 281 nucleotides whose sequence is that of the eDNA clone TS 103 (ref 51) and folds into a defined secondary structure identical to a Pol III derived transcript; 2: a single RNA strand of 302 nucleotides whose sequence is identical to that of a clone isolated from the RPE of a human eye with geographic atrophy that folds into a defined secondary structure identical to a Pol III derived transcript; or 3: a fully complementary dsRNA version of this 302 nucleotide long sequence that mimics a Pol II derived transcript) was injected at 0.3 mg/ml. Cellpermuting cholesterol conjugated-B1/B2 antisense oligonucleotides (as) (5'-TCAGATCTCGTTACGGATGGTTGTGA-3') or cholesterol conjugated-control as (5'-TTTGGATACAGCTGTTGAGTGA-3') or cholesterol conjugated-control as (5'-TTTGGATACAGCTGTTGAGTGA-3') (both from Integrated DNA Technologies) were injected (2 μg in 1 μL) 10 days after AAV1-BEST1-Cre was injected in Dicer1−/− mice.

**Isolation of dsRNA.**

Human eyes were stored in RNAlater (Ambion). Tissue extracts were prepared by lysis in buffer containing 50 mM Tris-HCl, pH 8, 150 mM NaCl, 1% Nonidet P-40, protease and phosphatase inhibitors (complete mini EDTA-free, protease inhibitor and phosphatase inhibitor cocktail tablets, Roche), and RNase inhibitor (SUPERNase-In, Ambion). After homogenization using bullet blender (Nextadvance) and centrifugation, immunoprecipitations were performed by adding 40 μg of mouse antibody against dsRNA (clone J2) for 16 h at 4° C. Immunocomplexes were collected on protein A/G agarose (Thermo Scientific) and dsRNA species...
were separated and isolated using Trizol (Invitrogen) according to the manufacturer’s instructions.

Ligation of dsRNA and Anchor Primer. An anchor primer (PC3-17 loop, 5'-p-CCGGGGATCCCGCCGATCC-3') was ligated to dsRNA (200-400 ng) in 50 mM HEPES/NaOH, pH 8 (vWR), 18 mM MgCl₂, 0.01% BSA (Fisher Scientific), 1 mM ATP (Roche), 3 mM DTT (Fluka), 10% DMSO (Finnzymes), 20% PEG 6000 (Alfa Aesar), and 30U T4 RNA ligase (Ambion). Ligation was performed at 37°C for 16 h, and ligated dsRNA was purified by MinElute Gel extraction columns (Qiagen).

Sequence-Independent cDNA Synthesis. After denaturation, ligated dsRNA was reverse transcribed in a RT reaction containing 50 mM Tris-HCl, pH 8.3, 10 mM MgCl₂, 70 mM KCl, 30 mM β-mercaptoethanol, 1 mM dNTPs and 15U cloned AMV reverse transcriptase (Invitrogen). The mixture was incubated in a thermal cycler (Eppendorf) at 42°C for 45 min followed by 55°C for 15 min.

Polymerase Chain Reaction (PCR) Amplification. Amplification of cDNA was performed using primer PC2 (5'-p-CCCGGCTGAGAGAACTTACG-3', IDT) in a reaction buffer containing 5 µL cDNA and 40 µL Platinum PCR SuperMix (Invitrogen). The PCR cycling parameters consisted of one step of 72°C for 1 min to fill incomplete cDNA ends and produce intact DNA, followed by one step of initial denaturation (94°C, 2 min), 39 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min, and a final extension step of 72°C for 10 min. In vitro transcribed dsRNAs of varying lengths (325 bp, 1 and 2 kb) were used as positive controls.

Cloning and Sequencing. The amplified cDNA products were incubated with 1 µL calf intestinal alkaline phosphatase (Invitrogen) at 37°C for 5 min to remove the 5'-phosphate group, separated on a low-melting point agarose gel (1%) and purified using Qiagen gel extraction kit (Qiagen). The purified dephosphorylated cDNA fragments were cloned in TOPO TA Cloning Vector (Invitrogen) and sequenced using M13 forward (5'-p-CCGGGGATCCCGCCGATCC-3') and reverse (5'-CTTTAATCCCAGCACTT-3') and reverse sequencing primers. The sequences have been deposited in GenBank under the accession numbers HIN176584 and HIN176585.

Alu RNA Synthesis. The present inventors synthesized two Alu RNAs: a 281 nt Alu sequence originating from the cDNA clone T8 103 which is known to be expressed in human cells and a 302 nt Alu sequence isolated from the RPE of a human eye with geographic atrophy. Both of these Alu RNAs were synthesized using a RNA polymerase T7 promoter and runoff transcription followed by gel purification as previously described. This yields single stranded RNAs that fold into a defined secondary structure identical to Pol III derived transcripts. The present inventors also synthesized a fully complemented dsRNA form (resembling a Pol II derived transcript) of the 302 nt human geographic atrophy Alu using linearized pCRII TOPO plasmid templates using 17 or SP6 RNA polymerases (MegaScript, Ambion) according to the manufacturer’s recommendations. After purification, equal molar amount of each transcript was combined and heated at 95°C for 1 min, cooled and then annealed at room temperature for 24 h. The Alu dsRNA was precipitated, suspended in water and analyzed on 1.4% non-denaturing agarose gel using the single-stranded complementary strands as controls.

Real-Time PCR. Total RNA was extracted from tissues or cells using Trizol reagent (Invitrogen) according to manufacturer’s recommendations and were treated with RNase free DNase (Ambion). Total RNA (1 µg) was reverse transcribed as previously described using qScript cDNA SuperMix (Quanta Biosciences). The RT products (cDNA) were amplified by real-time quantitative PCR (Applied Biosystems 7900 HT Fast Real-Time PCR system) with Power SYBR green Master Mix. Oligonucleotide primers specific for Dicer1 (forward 5'-CCCGGCGTCGAGAGAACTTACG-3' and reverse 5'-CTGTAACCTTGCAACACACCTTTAAA-3'), DROSHA (forward 5'-GAAGACAGTTCACCCCCGATTG-3' and reverse 5'-GCTAACCAGTTCGAGGCGTACT-3'), DCGR8 (forward 5'-TCTGTCCTTCATTGCGGTGACT-3' and reverse 5'-CCAAACTCCTCCGAAAAG-3'), EIF2C2 (forward 5'-GAACGGAGAGTCTACGAGATC-3' and reverse 5'-CCCGCGTCTCCTCGAGAATC-3'), human 18S rRNA (forward 5'-CCGACAGTTGACACCCGGAAGGAGA-3' and reverse 5'-GGTGCTTTACGTTTCAAGG-3'), and reverse 5'-GGTCTCGTCCTCGGTGACTG-3'). LINE 1.1.3 (ORF2) (forward 5'-CGGTAATTCCCTTGCTCAAGATA-3' and reverse 5'-TGATCGGCTTAACCTGTTGAG-3'), HERV-W1 (forward 5'-GGCCGTGTAATGCACCAGTAGTCC-3' and reverse 5'-GGGAGCCCTCATCTCCACT-3'), human RNA-associated Y3 (hY3) (forward 5'-CCCGAGTTCAGTGTGTATTCA-3' and reverse 5'-GGAG1GGGA-3'), 7SL (forward 5'-CCGGCATCAATATGCGTACCT-3' and reverse 5'-CATGATCGACGGAGGAGGAGTTTT-3'), B2 (forward 5'-TGCCTTTAATTTCCGAGCATT-3' and reverse 5'-GCTGCTCAACAAGGTTGAA-3'), B2 (forward 5'-GAGTTCGAAATCTCCGAGCAGT-3' and reverse 5'-AAGAGCCTGAGTTCGGTCAAG-3'), cytoplasmic B2 (forward 5'-GGGCTGTACATTTGCTTTT-3' and reverse 5'-GGGCTGTACATTTGCTTTT-3'), Dicer1 (forward 5'-CCCAACGGGATGTCAGTGT-3' and reverse 5'-TGGAGGGAGGAGGAGGAGGAGTTTT-3'), mouse 18S rRNA (forward 5'-TCCCTGCTGGCCTGCTGTCGTTTTT-3', and reverse 5'-GCTGCTCATGTCGTTTTT-3'), and reverse 5'-GGCCTGTTACATTGCTTT-3', and reverse 5'-GGCCTGTTACATTGCTTT-3'). The QPCR cycling conditions were 50°C for 2 min, 95°C for 10 min followed by 40 cycles of a two-step amplification program (95°C for 15 s and 58°C C. for 1 min). At the end of the amplification, melting curve analysis was applied using the dissociation protocol from the Sequence Detection system to exclude contamination with unspecific PCR products. The PCR products were confirmed by agarose gel and showed only one specific band of the predicted size. For negative controls, no RT products were used as templates in the QPCR and verified by the absence of gel-detected bands. Relative expressions of target genes were determined by the 2^-ΔΔCT method. miRNA PCR. miRNA abundance was quantified using the All-in-One™ miRNA qRT-PCR Detection Kit (GeneCopoeia). Briefly, total RNA was polyadenylated and reverse transcribed using
a poly dT-adaptor primer. Quantitative RT-PCR was carried out using a miRNA-specific forward primer and universal reverse primer. PCR products were subjected to dissociation curve and gel electrophoresis analyses to ensure that single, mature miRNA products were amplified. Data were normalized to ACTB levels. The forward primers for the miRNAs were as follows: miR-184 (5'-TGGACGGAGAACTGAGGAGTTTTGAC-3'); miR-221/222 (5'-AGCTCACATGGCTACTGGTGT-3'); miR-204/211 (5'-TTGCCCTTTGTCATCAGGTGTGGC-3'); miR-877 (5'-GTAGAGGAGATCCGAGGGTGTTTGAC-3'); miR-320a (5'-AAAAGCTCGGTGAGAGGCGCA-3'); miR-484 (5'-TCAGGTCAGCCCTCCTCCCGAGA-3'); let-7a (5'-TGAGATAAGAGGTTATGTT-3'). The reverse primers were proprietary (GeneCopoeia). The primers for ACTB were forward (5'-TGGATCAGCAAGCAGGAGTTTTGAC-3') and reverse (5'-TGGACGGAGAACTGAGGAGTTTTGAC-3').

The RNAs were crosslinked to the membranes by ultraviolet irradiation and baked at 80°C. For visualization of U6 RNA, U6 RNA probes were synthesized by PCR amplification of a 7SL RNA plasmid (pAlu, pCDNA3.1/Dicer1-FLAG, pCDNA3.1, Dicer1 antisense oligonucleotide (as) (5'-GCUGAC-CTTTTGTGCTCUCA-3'), B1/B2 as (5'-TCAGATCCTGGTTGATGGTTTGGA-3'), control (for Dicer1 and B1/B2) as (5'-TTGCTACGATCGCTGTTGATGGTTTGGA-3'), Alu as (5'-CCGCGGTTCAGCCAGTGATGGTTTGGA-3'), U6 as (5'-CCCGGGTTCAGCCAGTGATGGTTTGGA-3'). The RNAs were crosslinked to the membranes by ultraviolet irradiation and baked at 80°C. For visualization of U6 RNA, U6 RNA probes were synthesized by PCR amplification of a 7SL RNA plasmid (pAlu, pCDNA3.1/Dicer1-FLAG, pCDNA3.1, Dicer1 antisense oligonucleotide (as) (5'-GCUGAC-CTTTTGTGCTCUCA-3'), B1/B2 as (5'-TCAGATCCTGGTTGATGGTTTGGA-3'), control (for Dicer1 and B1/B2) as (5'-TTGCTACGATCGCTGTTGATGGTTTGGA-3'), Alu as (5'-CCGCGGTTCAGCCAGTGATGGTTTGGA-3'), U6 as (5'-CCCGGGTTCAGCCAGTGATGGTTTGGA-3'). Western Blot.

Tissues were homogenized in lysis buffer (10 mM Tris base, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.5% NP-40, protease and phosphatase inhibitor cocktail (Roche)). Protein concentrations were determined using a Bradford assay kit (Bio-Rad) with bovine serum albumin as a standard. Proteins (40-100 µg) were run on 4-12% Novex Bis-Tris gels (Invitrogen). The transferred membranes were blocked for 1 h at RT and incubated with antibodies against Dicer1 (1:1,000, ref. 85; or 1:200, Santa Cruz Biotechnology) at 4°C overnight. Protein loading was assessed by immuno blotting using an anti-Tubulin antibody (1:1,000; Sigma-Aldrich). The secondary antibodies were used (1:5,000) for 1 h at RT. The signal was visualized by enhanced chemiluminescence (ECL Plus) and captured by VisionWorksLS Image Acquisition and Analysis software (Version 6.7.2, UVP, LLC). Densitometry analysis was performed using ImageJ (NIH). The value of 1 was arbitrarily assigned for normal eye samples. Dicer1 Cleavage.

The ability of Dicer1 to cleave Alu RNA was tested using Reagent Human Dicer Enzyme Kit (Genlantis) according to the manufacturer's instructions. The products of the digestion were purified for the in vivo injection using RNA Purification Column (Genlantis).

Cell Culture.

All cell lines were cultured at 37°C and 5% CO2. Primary mouse RPE cells were isolated as previously described35 and grown in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% FBS and standard antibiotics concentrations. Primary human RPE cells were isolated as previously described28 and maintained in DMEM supplemented with 20% FBS and antibiotics. Parental HCT116 and isogenic Dicer1 cells25 were cultured in McCoy's 5A medium supplemented with 10% FBS. Transient Transfection.

Human and mouse RPE cells were transfected with pLC19, pAlu, pCDNA3.1/Dicer1-FLAG, pCDNA3.1, Dicer1 antisense oligonucleotide (as) (5'-GCUGACGCTTTTGTGCTCUCA-3'), B1/B2 as (5'-TCAGATCCTGGTTGATGGTTTGGA-3'), control (for Dicer1 and B1/B2) as (5'-TTGCTACGATCGCTGTTGATGGTTTGGA-3'), Alu as (5'-CCGCGGTTCAGCCAGTGATGGTTTGGA-3'), U6 as (5'-CCCGGGTTCAGCCAGTGATGGTTTGGA-3'). The RNAs were crosslinked to the membranes by ultraviolet irradiation and baked at 80°C. For visualization of U6 RNA, U6 RNA probes were synthesized by PCR amplification of a 7SL RNA plasmid (pAlu, pCDNA3.1/Dicer1-FLAG, pCDNA3.1, Dicer1 antisense oligonucleotide (as) (5'-GCUGACGCTTTTGTGCTCUCA-3'), B1/B2 as (5'-TCAGATCCTGGTTGATGGTTTGGA-3'), control (for Dicer1 and B1/B2) as (5'-TTGCTACGATCGCTGTTGATGGTTTGGA-3'), Alu as (5'-CCGCGGTTCAGCCAGTGATGGTTTGGA-3'), U6 as (5'-CCCGGGTTCAGCCAGTGATGGTTTGGA-3'). Lipofectamine 2000 (Invitrogen) or Oligofectamine (Invitrogen) according to the manufacturer's instructions. Adenoviral Infection.

Cells were plated at density of 15×104/cm2 and after 16 h, at approximately 50% confluence, were infected with AdCre or AdNull (Vector Laboratories) with a multiplicity of infection of 1,000. RNA Polymerase Inhibition.

Human RPE cells were transfected with Dicer1 or control antisense oligonucleotides using Lipofectamine 2000. After a change of medium at 6 h, the cells were incubated with 45 µM tagetitoxin (Epigen Technologies, Tagetin) or 10 µg/ml a-amanitin (Sigma-Aldrich) and the total RNA was collected after 24 h. Cell Viability.

MTS assays were performed using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega) in accordance to the manufacturer's instructions.
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Caspase-3 Activity.
Sub-confluent human RPE cells were treated with PBS or Alu RNA at different concentrations in 2% FBS medium for 8 h. The caspase-3 activity was measured using Caspase-3 Fluorimetric Assay (R&D Systems) according to the manufacturer’s instructions.

Oxidative Stress.
Confluent human RPE cells were exposed to hydrogen peroxide (0-2 mM, Fisher Scientific).

Statistics.
Results are expressed as mean±SEM, with P<0.05 considered statistically significant. Differences between groups were compared by using Mann-Whitney U test or Student t test, as appropriate, and 2-tailed P values are reported.

Throughout this document, various references are mentioned. All such references are incorporated herein by reference, including the references set forth in the following list:

REFERENCES


It will be understood that various details of the presently disclosed subject matter can be changed without departing from the scope of the subject matter disclosed herein. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.
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1 5 10 15
Pro Asn Pro Gly Leu Ile Leu Gln Ala Leu Thr Leu Ser Asn Ala Ser
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Asp Gly Phe Asn Leu Glu Arg Arg Met Leu Gly Asp Ser Phe Leu
35 40 45
Lys His Ala Ile Thr Thr Tyr Leu Phe Cys Thr Tyr Pro Asp Ala His
50 55 60
Glu Gly Arg Leu Ser Tyr Met Arg Ser Lys Lys Val Ser Asn Cys Asn
65 70 75 80
Leu Tyr Arg Leu Gly Lys Lys Leu Pro Ser Arg Met Val Val
85 90 95
Ser Ile Phe Asp Pro Pro Val Asn Thr Leu Pro Pro Gly Tyr Val Val
100 105 110
Asn Gln Asp Lys Ser Asn Thr Asp Asp Lys Asn Met Thr Lys Arg
115 120 125
Lys Asp Cys Met Leu Ala Asn Gly Leu Asp Glu Asp Tyr Glu Glu
130 135 140
Glu Asp Glu Glu Glu Ser Leu Met Trp Arg Ala Pro Lys Glu Glu
145 150 155 160
Ala Asp Tyr Glu Asp Asp Phe Leu Glu Tyr Asp Glu His Ile Arg
165 170 175
Phe Ile Asp Asn Met Leu Met Gly Ser Gly Ala Phe Val Lys Lys Ile
180 185 190
Ser Leu Ser Pro Phe Ser Thr Asp Ser Ala Tyr Glu Trp Lys Met
195 200 205
Pro Lys Lys Ser Ser Leu Gly Ser Met Pro Phe Ser Ser Asp Phe Glu
210 215 220
Asp Phe Asp Tyr Ser Ser Trp Asp Ala Met Cys Tyr Leu Asp Pro Ser
225 230 235 240
Lys Ala Val Glu Asp Phe Val Val Gly Phe Trp Asn Pro Ser
245 250 255
Glu Glu Asn Cys Gly Val Asp Thr Gly Lys Gin Ser Ile Ser Tyr Asp
260 265 270
Leu His Thr Glu Gin Cys Ile Ala Asp Asp Ser Ser Ala Asp Cys Val
275 280 285
Glu Ala Leu Leu Gly Cys Tyr Leu Thr Ser Cys Gly Glu Arg Ala Ala
290 295 300
Gln Leu Phe Leu Cys Ser Leu Gly Leu Lys Val Leu Pro Val Ile Lys
305 310 315 320
Arg Thr Asp Arg Glu Lys Ala Leu Cys Pro Thr Arg Glu Asn Phe Asn
325 330 335
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340 345 350
<210> SEQ ID NO 11
<211> LENGTH: 1318
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

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Gly Gly Pro Arg Val Thr Ile Asn Thr Ala Ile Gly His Ile Asn Arg
20    25    30
Tyr Cys Ala Arg Leu Pro Ser Asp Pro Phe Thr His Leu Ala Pro Lys
35    40    45
Cys Arg Thr Arg Glu Leu Pro Asp Gly Thr Phe Tyr Ser Thr Leu Tyr
50    55    60
Leu Pro Ile Asn Ser Pro Leu Arg Ala Ser Ile Val Gly Pro Pro Met
Ser Cys Val Arg Leu Ala Glu Arg Val Val Ala Leu Ile Cys Cys Glu
Lys Leu His Lys Ile Gly Glu Leu Asp Asp His Leu Met Pro Val Gly
Lys Glu Thr Val Lys Tyr Glu Glu Leu Asp Leu His Asp Glu Glu
Glu Thr Ser Val Pro Gly Arg Pro Gly Ser Thr Lys Arg Arg Glu Cys
Tyr Pro Lys Ala Ile Pro Glu Cys Leu Arg Asp Ser Tyr Pro Arg Pro
Asp Gln Pro Cys Tyr Leu Tyr Val Ile Gly Met Val Leu Thr Thr Pro
Leu Pro Asp Glu Leu Asn Phe Arg Arg Arg Leu Tyr Pro Pro Glu
Asp Thr Thr Arg Cys Phe Gly Ile Leu Thr Ala Lys Pro Ile Pro Gln
Ile Pro His Phe Pro Val Tyr Thr Arg Ser Gly Glu Val Thr Ile Ser
Ile Glu Leu Lys Ser Ser Gly Phe Met Leu Ser Leu Gln Met Leu Glu
Leu Ile Thr Arg Leu His Gln Tyr Ile Phe Ser His Ile Leu Arg Leu
Glu Lys Pro Ala Leu Glu Phe Lys Pro Thr Asp Ala Asp Ser Ala Tyr
Cys Val Leu Pro Leu Asn Val Val Asn Asp Ser Thr Leu Asp Ile
Asp Phe Lys Phe Met Glu Asp Ile Glu Lys Ser Glu Ala Arg Ile Gly
Ile Pro Ser Thr Lys Tyr Thr Tyr Glu Thr Pro Phe Val Phe Lys Leu
Glu Asp Tyr Gin Asp Ala Val Ile Ile Pro Arg Tyr Arg Asn Phe Asp
Gln Pro His Arg Phe Tyr Val Ala Asp Val Tyr Thr Asp Leu Thr Pro
Leu Ser Lys Phe Pro Ser Pro Glu Tyr Glu Thr Phe Ala Glu Tyr
Lys Thr Lys Tyr Asn Leu Asp Leu Thr Asn Leu Asn Gin Pro Leu Leu
Asp Val Asp His Thr Ser Ser Arg Leu Asn Leu Leu Thr Pro Arg His
Leu Asn Gln Lys Gly Lys Ala Leu Pro Leu Ser Ser Ala Glu Lys Arg
Lys Ala Lys Trp Glu Ser Leu Gin Asn Lys Gin Ile Leu Val Pro Glu
Leu Cys Ala Ile His Pro Ile Pro Ala Ser Leu Trp Arg Lys Ala Val
Cys Leu Pro Ser Ile Leu Tyr Arg Leu His Cys Leu Leu Thr Ala Glu
Glu Leu Arg Ala Gin Thr Ala Ser Asp Ala Gly Val Gly Val Arg Ser
Leu Pro Ala Asp Phe Arg Tyr Pro Asn Leu Asp Phe Gly Trp Lys Lys
Ser Ile Asp Ser Lys Ser Phe Ile Ser Ile Ser Asn Ser Ser Ser Ser Ala
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515 520 525
Ala His Gln Gly Ala Asn Arg Thr Ser Ser Leu Glu Asn His Asp Gln
530 535 540
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565 570 575
Asn Gln Asn Leu Ala Asn Gly Ser Tyr Asp Leu Ala Asn Arg Asp Phe
580 585 590
Cys Gln Gly Asn Leu Asn Tyr Tyr Lys Gin Gin Ile Pro Val Gin
595 600 605
Pro Thr Thr Ser Tyr Ser Ile Gin Asn Leu Tyr Ser Tyr Glu Asn Gin
610 615 620
Pro Gin Pro Ser Asp Glu Cys Thr Leu Ser Asn Lys Tyr Leu Asp
625 630 635 640
Gly Asn Ala Asn Lys Ser Thr Ser Asp Gly Ser Pro Val Met Ala Val
645 650 655
Met Pro Gly Thr Thr Asp Thr Ile Gin Val Leu Lys Gly Arg Met Asp
660 665 670
Ser Glu Gin Ser Pro Ser Ile Gly Tyr Ser Ser Arg Thr Leu Gly Pro
675 680 685
Asn Pro Gly Leu Ile Leu Gin Ala Leu Thr Leu Ser Asn Ala Asn Ser Asp
690 695 700
Gly Phe Asn Leu Glu Arg Leu Glu Met Leu Gly Asp Ser Phe Leu Lys
705 710 715 720
His Ala Thr Thr Tyr Leu Phe Cys Thr Tyr Pro Asp Ala His Glu
725 730 735
Gly Arg Leu Ser Tyr Met Arg Ser Lys Lys Val Ser Asn Cys Asn Leu
740 745 750
Tyr Arg Leu Gly Lys Lys Gin Gin Leu Pro Ser Arg Met Val Val Ser
755 760 765
Ile Phe Asp Pro Pro Val Asp Leu Leu Gly Tyr Pro Gly Tyr Val Val Asn
770 775 780
Gln Asp Lys Ser Asn Thr Asp Lys Trp Glu Lys Asp Glu Met Thr Lys
785 790 795 800
Asp Cys Met Leu Ala Asn Gly Leu Asp Glu Asp Tyr Glu Glu Glu
805 810 815
Asp Glu Glu Glu Ser Leu Met Trp Arg Ala Pro Lys Glu Glu Ala
820 825 830
Asp Tyr Glu Asp Phe Leu Glu Tyr Asp Gin Glu His Ile Arg Phe
835 840 845
Ile Asp Asn Met Leu Met Gly Ser Gly Ala Phe Val Lys Lys Ile Ser
850 855 860
Leu Ser Pro Phe Ser Thr Asp Ser Ala Tyr Glu Trp Lys Met Pro
865 870 875 880
Lys Lys Ser Ser Leu Gly Ser Met Pro Phe Ser Ser Asp Phe Glu Asp
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

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Tyr Leu Leu Gln Ala Phe Thr His Ala Ser Tyr His Tyr Asn Thr Ile 20 25 30
Thr Asp Cys Tyr Glu Arg Leu Glu Phe Leu Gly Lys Ala Ile Leu Asp 35 40 45
Tyr Leu Ile Thr Lys His Tyr Glu Asp Arg Lys His Ser Pro 50 55 60
Gly Val Leu Thr Asp Leu Arg Ser Ala Leu Val Asn Thr Ile Phe 65 70 75 80
Ala Ser Leu Ala Val Lys Tyr Asp Tyr His Tyr Lys Tyr Phe Lys Ala Val 95 100 105 110
Ser Pro Glu Leu Phe His Val Ile Asp Asp Phe Val Glu Phe Gly Leu 115 120 125
Glu Lys Asn Glu Met Glu Gly Met Asp Ser Glu Leu Arg Arg Ser Glu 130 135 140
Glu Asp Glu Glu Lys Glu Glu Asp Ile Glu Val Pro Lys Ala Met Gly 145 155 160
Asp Ile Phe Glu Ser Leu Ala Gly Ala Ile Tyr Met Asp Ser Gly Met 165 175
Ser Leu Glu Thr Val Tyr Glu Val Tyr Tyr Pro Met Arg Pro Leu 180 185 190
Ile Glu Lys Phe Ser Ala Asn Val Pro Arg Ser Pro Val Arg Glu Leu 195 200 205
Leu Glu Met Glu Pro Glu Thr Ala Lys Phe Ser Pro Ala Glu Arg Thr 210 215 220
Tyr Asp Gly Lys Val Arg Val Thr Val Glu Val Val Gly Lys Gly Lys 225 230 235 240
Phe Lys Gly Val Gly Arg Ser Tyr Arg Ile Ala Lys Ser Ala Ala 245 250 255
Arg Arg Ala Leu Arg Ser Leu Lys Ala Asn Glu Pro Glu Val Pro Asn 260 265 270

Ser

<210> SEQ ID NO 13
<211> LENGTH: 247
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Phe Glu Asn Phe Glu Lys Ile Asn Tyr Arg Phe Lys Asn Lys Ala 1 5 10 15
Tyr Leu Leu Gln Ala Phe Thr His Ala Ser Tyr His Tyr Asn Thr Ile 20 25 30
Thr Asp Cys Tyr Glu Arg Leu Glu Phe Leu Gly Lys Ala Ile Leu Asp 35 40 45
Tyr Leu Ile Thr Lys His Leu Tyr Glu Asp Arg Glu His Ser Pro 50 55 60
Gly Val Leu Thr Asp Leu Arg Ser Ala Leu Val Asn Asn Thr Ile Phe 65 70 75 80

Ala Ser Leu Ala Val Lys Tyr Tyr Asp Tyr His Lys Tyr Phe Lys Ala Val 85 90 95

Ser Pro Glu Leu Phe His Val Ile Asp Asp Phe Val Gln Phe Glu Leu 100 105 110

Glu Lys Asn Glu Met Gln Gly Met Asp Ser Glu Leu Arg Arg Ser Glu 115 120 125

Glu Asp Glu Lys Glu Glu Asp Ile Glu Val Pro Lys Ala Met Gly 130 135 140

Asp Ile Phe Glu Ser Leu Ala Gly Ala Ile Tyr Met Asp Ser Gly Met 145 150 155 160

Ser Leu Glu Thr Val Trp Gln Val Tyr Tyr Pro Met Met Arg Pro Leu 165 170 175

Ile Glu Lys Phe Ser Ala Asn Val Pro Arg Ser Pro Val Arg Glu Leu 180 185 190

Leu Glu Met Glu Pro Glu Thr Ala Lys Phe Ser Pro Ala Glu Arg Thr 195 200 205

Tyr Asp Gly Lys Val Arg Val Val Glu Val Gly Lys Gly Lys 210 215 220

Phe Lys Gly Val Gly Arg Ser Tyr Arg Ile Ala Lys Ser Ala Ala Ala 225 230 235 240

Arg Arg Ala Leu Arg Ser Leu 245

<210> SEQ ID NO: 14
<211> LENGTH: 234
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: Homo sapiens

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Tyr Leu Leu Gln Ala Phe Thr His Ala Ser Tyr His Tyr Asn Thr Ile 20 25 30

Thr Asp Cys Tyr Gln Arg Leu Glu Phe Leu Gly Asp Ala Ile Leu Asp 35 40 45

Tyr Leu Ile Thr Lys His Leu Tyr Glu Asp Pro Arg Gln His Ser Pro 50 55 60

Gly Val Leu Thr Asp Leu Arg Ser Ala Leu Val Asn Asn Thr Ile Phe 65 70 75 80

Ala Ser Leu Ala Val Lys Tyr Tyr Asp Tyr His Lys Tyr Phe Lys Ala Val 85 90 95

Ser Pro Glu Leu Phe His Val Ile Asp Asp Phe Val Gln Phe Glu Leu 100 105 110

Glu Lys Asn Glu Met Gln Gly Met Asp Glu Asp Ile Glu Val Pro Lys 115 120 125

Ala Met Gly Asp Ile Phe Glu Ser Leu Ala Gly Ala Ile Tyr Met Asp 130 135 140

Ser Gly Met Ser Leu Glu Thr Val Trp Gln Val Tyr Tyr Pro Met Met 145 150 155 160

Arg Pro Leu Ile Glu Lys Phe Ser Ala Asn Val Pro Arg Ser Pro Val 165 170 175

Arg Glu Leu Leu Glu Met Glu Pro Glu Thr Ala Lys Phe Ser Pro Ala
<210> SEQ ID NO 15
<211> LENGTH: 550
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15
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Gly Pro Asn Pro Gly Leu Ile Leu Gln Ala Leu Thr Leu Ser Asn Ala 20 25 30
Ser Asp Gly Phe Asn Leu Glu Arg Leu Glu Met Leu Gly Asp Ser Phe 35 40 45
Leu Lys His Ala Ile Thr Thr Tyr Leu Phe Cys Thr Tyr Pro Asp Ala 50 55 60
His Glu Gly Arg Leu Ser Tyr Met Arg Ser Lys Val Ser Asn Cys 65 70 75 80
Asn Leu Tyr Arg Leu Gly Lys Lys Gly Leu Pro Ser Arg Met Val 85 90 95
Val Ser Ile Phe Asp Pro Pro Val Asn Trp Leu Pro Pro Gly Tyr Val 100 105 110
Val Asn Gln Asp Lys Ser Asn Thr Lys Tyr Gln Lys Asp Glu Met 115 120 125
Thr Lys Asp Cys Met Leu Ala Asn Gly Lys Leu Asp Glu Asp Tyr Glu 130 135 140
Glu Glu Asp Glu Glu Glu Ser Leu Met Trp Arg Ala Pro Lys Glu 145 150 155 160
Glu Ala Asp Tyr Glu Asp Phe Leu Glu Tyr Asp Glu Glu His Ile 165 170 175
Arg Phe Ile Asp Ann Met Leu Met Gly Ser Gly Ala Phe Val Lys Lys 180 185 190
Ile Ser Leu Ser Pro Phe Ser Thr Thr Asp Ser Ala Tyr Glu Trp Lys 195 200 205
Met Pro Lys Lys Ser Ser Leu Gly Ser Met Pro Phe Ser Ser Asp Phe 210 215 220
Glu Asp Phe Asp Tyr Ser Ser Trp Asp Ala Met Cys Tyr Leu Asp Pro 225 230 235 240
Ser Lys Ala Val Glu Asp Asp Phe Val Val Gly Phe Trp Asn Pro 245 250 255
Ser Glu Glu Asn Cys Gly Val Asp Thr Gly Lys Glu Ser Ile Ser Tyr 260 265 270
Asp Leu His Thr Glu Gln Cys Ile Ala Asp Lys Ser Ile Ala Asp Cys 275 280 285
Val Glu Ala Leu Leu Gly Cys Tyr Leu Thr Ser Cys Gly Glu Arg Ala 290 295 300
Ala Gln Leu Phe Leu Cys Ser Leu Gly Leu Val Leu Pro Val Ile 305 310 315 320
Lys Arg Thr Asp Arg Glu Lys Ala Cys Pro Thr Arg Glu Asn Phe 1 5 10 15
Asn Ser Gln Gln Lys Asn Leu Ser Val Ser Cys Ala Ala Ala Ser Val 20 25 30 35
Ala Ser Ser Arg Ser Ser Val Leu Lys Asp Ser Glu Tyr Gly Cys Leu 30 35 40 45
Lys Ile Pro Pro Arg Cys Met Phe Asp His Pro Asp Ala Asp Lys Thr 45 50 55 60
Leu Asn His Leu Ile Ser Gly Phe Glu Asn Phe Glu Lys Lys Ile Asn 75 80 85 90
Tyr Arg Phe Lys Asn Lys Ala Tyr Leu Leu Glu Ala Phe Thr His Ala 105 110 115 120
Ser Tyr His Tyr Asn Thr Ile Thr Asp Cys Tyr Glu Arg Leu Glu Phe 125 130 135 140
Leu Gly Asp Ala Ile Leu Asp Tyr Leu Ile Thr Asp His Leu Tyr Glu 145 150 155 160
Asp Pro Arg Gln His Ser Pro Gly Val Leu Thr Asp Leu Arg Ser Ala 165 170 175 180
Leu Val Asn Asn Thr Ile Phe Ala Ser Leu Ala Val Lys Tyr Asp Tyr 185 190 195 200
His Lys Tyr Phe Lys Ala Val Ser Asp Glu Leu Phe His Val Ile Asp 205 210 215 220
Asp Phe Val Gln Gln Leu Glu Lys Asn Glu Met Glu Gly Met Asp 225 230 235 240
Ser Glu Leu Arg Arg Ser Glu Glu Asp Glu Lys Lys Glu Glu Asp Ile 245 250 255 260
Glu Val Pro Lys Ala Met Gly Asp Ile Phe Glu Ser Leu Ala Gly Ala 265 270 275 280
Ile Tyr Met Asp Ser Gly 285 290 295 300

<210> SEQ ID NO: 16
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 16
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Aasp Gly Phe Asn Leu Glu Arg Leu Glu Met Leu Gly Asp Ser Phe Leu 30 35 40 45
Lys His Ala Ile Thr Thr Tyr Leu Phe Cys Thr Tyr Pro Asp Ala His 50 55 60 65
Glu Gly Arg Leu Ser Tyr Met Arg Ser Lys Val Ser Asn Cys Asn 70 75 80 85
Leu Tyr Arg Leu Gly Lys Lys Gly Leu Pro Ser Arg Met Val Val 90 95 100 105
Ser Ile Phe Asp Pro Pro Val Asn Trp Leu Pro Pro Gly Tyr Val Val 110 115 120 125
Aasp Gln Asp Lys Ser Asn Thr Asp Lys Trp Glu Lys Asp Glu Met Thr 130 135 140 145
Lys Asp Cys Met Leu Ala Asn Gly Lys Leu Asp Glu Asp Tyr Glu Glu 150 155 160 165
Glu Asp Glu Glu Glu Ser Leu Met Trp Arg Ala Pro Lys Glu Glu
145 150 155 160
Glu Asp Tyr Glu Asp Asp Phe Leu Glu Tyr Asp Glu Glu His Ile Arg
165 170 175
Phe Ile Asp Asn Met Leu Met Met Gly Ser Gly Ala Phe Val Lys Lys Ile
180 185 190
Ser Leu Ser Pro Phe Ser Thr Thr Asp Ser Ala Tyr Glu Trp Lys Met
195 200 205
Pro Lys Lys Ser Ser Leu Gly Ser Met Pro Phe Ser Ser Asp Phe Glu
210 215 220
Asp Phe Asp Tyr Ser Ser Trp Asp Ala Met Cys Tyr Leu Asp Pro Ser
225 230 235 240
Lys Ala Val Glu Asp Asp Phe Val Val Gly Phe Trp Asp Pro Ser
245 250 255
Glu Glu Asn Cys Gly Val Asp Thr Gly Lys Glu Glu Ser Ile Ser Tyr Asp
260 265 270
Leu His Thr Glu Glu Cys Ile Ala Asp Tyr Ser Leu Ala Asp Cys Val
275 280 285
Glu Ala Leu Leu Gly Cys Tyr Leu Thr Ser Cys Gly Glu Arg Ala Ala
290 295 300
Gln Leu Phe Leu Cys Ser Leu Gly Leu Lys Val Leu Pro Val Ile Lys
305 310 315 320
Arg Thr Asp Arg Glu Lys Ala Leu Cys Pro Thr Arg Glu Asn Phe Asn
325 330 335
Ser Gln Gln Lys Asn Leu Ser Val Ser Cys Ala Ala Ala Ser Val Ala
340 345 350
Ser Ser Arg Ser Ser Val Leu Lys Asp Ser Glu Tyr Gln Cys Leu Lys
355 360 365
Ile Pro Pro Arg Cys Met Phe Asp His Pro Asp Ala Asp Phe Leu Thr Leu
370 375 380
Asn His Leu Ile Ser Gly Phe Glu Asn Phe Glu Lys Ile Asn Tyr
385 390 395 400
Arg Phe Lys Asn Lys Ala Tyr Leu Leu Gin Ala Phe Thr His Ala Ser
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Tyr His Tyr Asn Thr Ile Thr Asp Cys Tyr Gin Arg Leu Glu Phe Leu
420 425 430
Gly Asp Ala Ile Leu Asp Tyr Leu Ile Thr Lys His Leu Tyr Glu Asp
435 440 445
Pro Arg Glu His Ser Pro Gly Val Leu Thr Asp Leu Arg Ser Ala Leu
450 455 460
Val Asn Thr Ile Phe Ala Ser Leu Ala Val Lys Tyr Asp Tyr His
465 470 475 480
Lys Tyr Phe Lys Ala Val Ser Pro Glu Leu Phe His Val Ile Asp Asp
485 490 495
Asn Leu Glu Glu Asp Ala Val Ser Leu Ala Val Lys Tyr Asp Tyr His
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SEQ  ID  NO  17
LENGTH: 10323
TYPE: DNA
ORGANISM: Homo sapiens

SEQUENCE: 17

US 9,464,289 B2
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taatctcaag gtctccgctt gcaagcagta aatggaatgg aatggaatgg aatggaatgg 1920
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<210> SEQ ID NO 20
<211> LENGTH: 754
<212> TYPE: PRT
<213> ORGANISM: Giardia lamblia virus

<400> SEQUENCE: 20
Met His Ala Leu Gly His Cys Thr Val Val Thr Thr Arg Gly Pro
Ser His Trp Leu Leu Leu Leu Asp Thr His Leu Gly Thr Leu Pro Gly
Phe Lys Val Ser Ala Gly Arg Gly Leu Pro Ala Ala Glu Val Tyr Phe
Glu Ala Gly Pro Arg Val Ser Leu Ser Arg Thr Asp Ala Thr Ile Val
Ala Val Tyr Gln Ser Ile Leu Phe Gln Leu Leu Leu Gly Pro Thr Phe Pro

65  70  75  80

Ala Ser Trp Thr Glu Ile Gly Ala Thr Met Pro His Asn Glu Tyr Thr

85  90  95

Phe Pro Arg Phe Ile Ser Asn Pro Pro Gln Phe Ala Thr Leu Ala Phe

100 105 110

Leu Pro Leu Leu Ser Pro Thr Ser Pro Leu Asp Leu Arg Ala Leu Met

115 120 125

Val Thr Ala Gln Leu Met Cys Asp Ala Lys Arg Leu Ser Asp Glu Tyr

130 135 140

Thr Asp Tyr Ser Thr Leu Ser Ala Ser Leu His Gly Arg Met Val Ala

145 150 155 160

Thr Pro Glu Ile Ser Trp Ser Leu Tyr Val Val Leu Gly Ile Asp Ser

165 170 175

Thr Gln Thr Ser Leu Ser Tyr Phe Thr Arg Ala Asn Glu Ser Ile Thr

180 185 190

Tyr Met Arg Tyr Tyr Ala Thr Ala His Asn Ile His Leu Arg Ala Ala

195 200 205

Asp Leu Pro Leu Val Ala Val Arg Leu Asp Leu Lys Asp His

210 215 220

Gln Ile Pro Ala Pro Gly Ser Trp Asp Leu Ala Pro Lys Leu Arg

225 230 235 240

Phe Leu Pro Pro Glu Leu Cys Leu Leu Leu Pro Asp Glu Phe Asp Leu

245 250 255

Ile Arg Val Gln Ala Leu Gln Phe Leu Pro Glu Ile Ala Lys His Ile

260 265 270

Cys Asp Ile Gln Asn Thr Ile Cys Ala Leu Asp Lys Ser Phe Pro Asp

275 280 285

Cys Gly Arg Ile Gly Gly Glu Arg Tyr Phe Ala Ile Thr Ala Gly Leu

290 295 300

Arg Leu Asp Gln Gly Arg Gly Arg Leu Ala Gly Trp Arg Thr Pro

305 310 315 320

Phe Gly Pro Phe Gly Val Ser His Thr Asp Val Phe Gln Arg Leu Glu

325 330 335

Leu Leu Gly Asp Ala Val Leu Gly Phe Ile Val Thr Ala Arg Leu Leu

340 345 350

Cys Leu Phe Pro Asp Ala Ser Val Gly Thr Leu Val Glu Leu Lys Met

355 360 365

Glu Leu Val Arg Asn Glu Ala Leu Asn Tyr Leu Val Glu Thr Leu Gly

370 375 380

Leu Pro Gln Leu Ala Gln Phe Ser Asn Asn Leu Val Ala Lys Ser Lys

385 390 395 400

Thr Trp Ala Asp Met Tyr Glu Ile Val Gly Ser Ile Phe Thr Gly

405 410 415

Pro Asn Gly Ile Tyr Gly Cys Glu Glu Phe Leu Ala Lys Thr Leu Met

420 425 430

Ser Pro Glu His Ser Lys Thr Val Gly Ser Ala Cys Pro Asp Ala Val

435 440 445

Thr Lys Ala Ser Lys Arg Val Cys Met Gly Glu Ala Gly Ala His Glu

450 455 460

Phe Arg Ser Leu Val Asp Tyr Ala Cys Glu Gin Gly Ile Ser Val Phe

465 470 475 480
Cys Ser Ser Arg Val Ser Thr Met Phe Leu Glu Arg Leu Arg Asp Ile 485 490 495
Pro Ala Glu Asp Met Leu Asp Trp Tyr Leu Gly Ile Gln Phe Ser 500 505 510
His Arg Ser Gly Leu Ser Gly Pro Gly Gly Val Ser Val Ser Val Ile Asp 515 520 525
Ile Met Thr His Leu Ala Arg Gly Leu Trp Leu Gly Ser Pro Gly Phe 530 535 540
Tyr Val Glu Gln Gln Thr Asp Lys Asn Glu Ser Ala Cys Pro Pro Thr 545 550 555 560
Ile Pro Val Leu Tyr Ile Tyr His Arg Ser Val Glu Cys Pro Val Leu 565 570 575
Tyr Gly Ser Leu Thr Glu Thr Pro Thr Gly Pro Val Ala Ser Lys Val 580 585 590
Leu Ala Leu Tyr Glu Ile Leu Ala Tyr Gly Ser Ser Gly Gly 595 600 605
Lys His Ile Ala Ala Gln Thr Val Ser Arg Ser Leu Ala Val Pro Ile 610 615 620
Pro Ser Gly Thr Ile Pro Phe Leu Ile Arg Leu Gln Ile Ala Leu 625 630 635 640
Thr Pro His Val Tyr Gin Leu Leu Leu Gly Asp Ala Phe Leu 645 650 655
Lys Cys Ser Leu Ala Leu His Ala Leu His Ala Leu His Pro Thr Leu Thr 660 665 670
Glu Gly Ala Leu Thr Arg Met Arg Gin Ser Ala Glu Thr Asn Ser Val 675 680 685
Leu Gly Arg Leu Thr Lys Arg Phe Pro Ser Val Ser Glu Val Ile 690 695 700
Ile Glu Ser His Pro Lys Ile Gin Pro Asp Ser Lys Val Tyr Gly Asp 705 710 715 720
Thr Phe Glu Ala Ile Leu Ala Ala Ile Leu Ala Cys Gly Glu Glu 725 730 735
Ala Ala Gly Ala Phe Val Arg Glu His Val Leu Pro Gin Val Val Ala 740 745 750
Amp Ala

<210> SEQ ID NO 21
<211> LENGTH: 2265
<212> TYPE: DNA
<213> ORGANISM: Giardia lamblia virus
<400> SEQUENCE: 21
atgcagcttt tgggacagtg tgtgacacta gaggacattc caactggttg 60
tcttcttag acactcaact gggacacctg ccaggtttta aagttatgatc aggccgaggg 120
tctccgcg cagaggtgtca tttgagctcg ggtcgcaggg tgtctttctc tcgaactgat 180
gcaacctgag tagccgtaggtctagttctttcttc gctcgtgattagc 240
gtttactgtag aggattatag gctgtggact aatgtgcctgc 300
atatccgttg gacagactct tgtctctttc ccaggtttttactt tctagttgggca 360
tctctttctag aatggttcttg gtcggttcttg ccggttttctt cttctactactaaggtttgc 420
tcggactgtg gacagactct gctgtggact aatgtgcctgc 480
acctgtagtgctagatctag tgtctctttc ccaggtttttactt tctagttgggca 540
cttttacactt taccaagaac aataatattca ataacaatacata tgagatacta tgcaacagcc
cacaatttcc accgctcttc ggctttgcttg cagcagctcg attagacgat cttaaaagacc
acctgcgtgc tgcagatctt ccgcttgtgg cagcagtcag attagacgat
acctgtgtcttttgaggta aagctccttc tctcaagctcatt cattgctcttg cggagaacc
acctgcgtgttttgaggta aagctccttc tctcaagctcatt cattgctcttg cggagaacc
cacaacgtttg acacctttc ttttccacag ctcttcttgg gtttcgaccag taatgagatc

te107

tttttcggttc cgccattctc ctgcctcagc ctcacgagta gctgggacta caggcgcccg
acaccactcc cggctaattt ttttgttttttttta ttttccacag ctcttcttgg cggagaacc
acaccactcc cggctaattt ttttgttttttttta ttttccacag ctcttcttgg cggagaacc

<210> SEQ ID NO 22
<211> LENGTH: 91
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE: 
<223> OTHER INFORMATION: oligonucleotide
<400> SEQUENCE: 22

ccccgggttc ccaggtcttc ctgcctcagc ctcagcagta gctgggtcctc caggggcccg
acaccactc cggctaaatttt tttgttaatttt ttttccacag ctcttcttgg cggagaacc

<210> SEQ ID NO 23
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE: 

ccccggttc ccaggtcttc ctgcctcagc ctcagcagta gctgggtcctc caggggcccg
acaccactc cggctaaatttt tttgttaatttt ttttccacag ctcttcttgg cggagaacc

<210> SEQ ID NO 21
<211> LENGTH: 107
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE: 
<223> OTHER INFORMATION: oligonucleotide
<400> SEQUENCE: 21

cttttacactt taccaagaac aataatattca ataacaatacata tgagatacta tgcaacagcc
cacaatttcc accgctcttc ggctttgcttg cagcagctcg attagacgat cttaaaagacc
acctgcgtgc tgcagatctt ccgcttgtgg cagcagtcag attagacgat
<223> OTHER INFORMATION: Oligonucleotide

<400> SEQUENCE: 23

tgagtcagg agatcgagac catcccggc

<210> SEQ ID NO 24
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<222> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 24

tgagtcagg agatcgaaac catcccggc

<210> SEQ ID NO 25
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<222> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 25

tgagtcagg agttcgaaac catcccggc

<210> SEQ ID NO 26
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<222> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 26

tgagtcagg agttcgagac catcccggc

<210> SEQ ID NO 27
<211> LENGTH: 291
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<222> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 27

ggcgggccgc ggtggtctac ggtgtaact cccgaccttt gggagccgca ggcgggtgga 60
tcacagtgg tcggaggttc gagagcagcc tggccacact ggtgaacccc cgctctctact 120
aasatcaca aaatagccq gcrgtggtg gggcgctctg taaatccccc tatacgggag 180
gctgaggcag gagaatctgt tgaacccggg aggccgtgcgt tcgagtcgct cgagatcgcg 240
cacatgca cccgcctggg cacaagagcc gcgacttggt ctcaaaaaa a 291

<210> SEQ ID NO 28
<211> LENGTH: 302
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<222> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 28

ggcgggccgc aatgcgctac acctctaact cccgaccttt ggcaggtcgtg ggcggcccag 60
tcacagtgg tcggaggttc gcggacatcc tggctgacact ggtgaacccc cgctctctact 120
aasatcaca aaatagccq ggcggtgttg ggtaggtctg gtagctccgc ctactgggca 180
ggagaattg gtaacccctg ggcggcggg ttacggtgag tccagcctgg gccactgcac 240
tccagctgg gcctactggt gcagacttgg ctcaaaaaa aaacaggcga aaagaaaaa 300
aa 302
What is claimed is:

1. An isolated nucleotide molecule selected from:
   - a double-stranded RNA molecule that inhibits expression of Alu RNA, wherein a first strand of the double-stranded RNA comprises a sequence selected from SEQ ID NO: 1, 2, 3, 4, 5, and 6 and including about 19 to 25 nucleotides; and
   - a vector comprising an oligonucleotide that inhibits the expression of Alu RNA, comprising a sequence selected from SEQ ID NO: 22, 23, 24, and 25 and including about 29 to 100 nucleotides; and
   - a vector comprising an oligonucleotide that inhibits the expression of Alu RNA, consisting of the sequence of SEQ ID NO: 26.


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