Protection of Cells from \textit{Alu}-RNA-Induced Degeneration and Inhibitors for Protecting Cells

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PROTECTION OF CELLS FROM ALU-RNA-INDUCED DEGENERATION AND INHIBITORS FOR PROTECTING CELLS

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This patent is subject to a terminal disclaimer.

Related U.S. Application Data

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References Cited

U.S. PATENT DOCUMENTS

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(Continued)

ABSTRACT

A method of protecting a cell includes inhibiting an inflammasome, MyD88, IL-18, NLRD1, NLRD1, caspase-8, and/or NFκB of the cell. Administering an inhibitor of MyD88, IL-18, NLRD1, NLRD1, caspase-8, and/or NFκB can protect the cell from Alu-RNA-induced degeneration. Protecting a cell, such as a retinal pigment epithelium (RPE), can be therapeutically useful in the context of age-related macular degeneration and geographic atrophy.

25 Claims, 23 Drawing Sheets
References Cited

OTHER PUBLICATIONS


(56)

References Cited

OTHER PUBLICATIONS


Ko et al., The Role of TLR4 Activation in Photoreceptor Mitochondrial Oxidative Stress, IOVS, 2011, vol. 52, No. 8, 5824-5835.


Bulosan et al., “Inflammatory caspases are critical for enhanced cell death in the target tissue of Sjogren’s syndrome before disease onset”. Immunology and Cell Biology, vol. 87, No. 1, Oct. 21, 2008, pp. 81-90.


* cited by examiner
Figure 4
Figure 9
Figure 12

A

DICER1 AS / Control AS

Caspalux Bright-field Overlay

(1.0)

(17)

B

DICER1 AS / Alu AS

Caspalux Bright-field Overlay

(0.1)

(1.0)
Wild-type+pAlu

control inhibitor  Caspase-8 inhibitor 2 μg

Figure 14

Alu RNA + Z-FA-FMK

Alu RNA + Z-IETD-FMK

Figure 15
Figure 18

CD95-/-(Fas\textsuperscript{pr}) mice

pNull  pAlu

Figure 19

CD95-/-(Fas\textsuperscript{pr}) mice

vehicle  Alu RNA
CD95⁻/⁻ (Fas^{br}) mice

Figure 20

Faslg⁻/⁻ (Fas^{gl}) mice

Figure 21
Faslg-/-(Fasl<sup>gld</sup>) mice

vehicle | Alu RNA

Figure 22

Faslg-/-(Fasl<sup>gld</sup>) mice

vehicle | Recombinant IL-18

Figure 23
Figure 24

NfkB1 -/- mice

vehicle

Alu RNA
Figure 25

vehicle  Alu RNA
Figure 26
Dye Light 780 VAD-FMK

vehicle recombinant IL-18

baseline

6 hrs

24 hrs

Figure 27
Figure 28
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.

The presently-disclosed subject matter includes methods for identifying MyD88 inhibitors, and methods and compositions for inhibiting MyD88 and uses thereof. The presently-disclosed subject matter includes methods for identifying inflammasome inhibitors, including a component of an inflammasome, and methods and compositions for inhibiting an inflammasome and uses thereof. The presently-disclosed subject matter includes methods for identifying inhibitors of components of inflammasome, and methods and compositions for inhibiting a component of inflammasome and uses thereof. Components of inflammasome include, for example, NLRP3, PYCARD, and Caspase-1. The presently-disclosed subject matter includes methods for identifying IL-18 inhibitors, and methods and compositions for inhibiting IL-18 and uses thereof. The presently-disclosed subject matter includes methods for identifying VDAC1 and VDAC2 inhibitors, and methods and compositions for inhibiting VDAC1 and VDAC2 and uses thereof. The presently-disclosed subject matter includes methods for identifying caspase-8 inhibitors, and methods and compositions for inhibiting caspase-8 and uses thereof. The presently-disclosed subject matter includes methods for identifying NFkB inhibitors, and methods and compositions for inhibiting NFkB and uses thereof. Also provided are methods and compositions for imaging activated caspase-1 in an eye of a subject.

The presently-disclosed subject matter includes methods including inhibiting one or more of an inflammasome, MyD88, and IL-18 of a cell. In some embodiments, the presently-disclosed subject matter includes methods including inhibiting one or more of MyD88, IL-18, VDAC1, VDAC2, NfKb, caspase-8, caspase-1, NLRP-3, PYCARD, and an inflammasome, including a component of an inflammasome (e.g., caspase 1, NLRP-3, PYCARD) of a cell. In some embodiments, the presently-disclosed subject matter includes methods including administering one or more inhibitors selected from inhibitors of MyD88, IL-18, VDAC1, VDAC2, NfKb, caspase-8, caspase-1, NLRP-3, PYCARD, and an inflammasome, including a component of an inflammasome (e.g., caspase 1, NLRP-3, PYCARD).

In some embodiments of the method, the cell is selected from a RPE cell, a retinal photoreceptor cell, or a choroidal cell. In some embodiments, the cell is an RPE cell. In some embodiments, the cell is the cell of a subject. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having a condition of interest. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having age-related macular degeneration. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having geographic atrophy. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having geographic atrophy and the cell is an RPE cell. In some embodiments, a subject having age-related macular degeneration can be treated using methods and compositions as disclosed herein.
BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Alu RNA does not activate or function via toll-like receptors (TLRs) (A-E) pAlu, but not pNull, induces RPE degeneration in WT (A), Tlr3−/− (B), Tlr7−/− (C), Uner93b1 mt mice, which are functionally deficient in TLRs-3,7,9 (D), and Tlr4−/− mice (E). Representative images shown. n=8-12. Fundus photographs, top row; Flat mounts stained for zonula occludens-1 (ZO-1; red), bottom row. Degeneration outlined by blue arrowheads. Scale bars, 20 μm. (F) Stimulation of HEK293 cell lines expressing various TLRs with either of two different Alu RNA sequences does not elicit NF-κB activation. Positive (+) controls using TLR-specific ligands activated NF-κB. n=3. Data are represented as mean±SEM. See also FIG. 8.

FIG. 2. Alu RNA induces RPE degeneration via MyD88 (A) pAlu does not induce RPE degeneration in Myd88−/− mice. (B) pAlu-induced RPE degeneration in WT mice is inhibited by a MyD88 homodimerization peptide inhibitor (MyD88i), but not by a control peptide. (C) pAlu-induced RPE degeneration in WT mice is inhibited by cholesterol-conjugated MyD88 siRNA but not control siRNA. (D and E) siRNA targeting MyD88 (siMyD88) reduces target gene (D) and protein (E) abundance in mouse RPE cells compared to control siRNA. n=3. *p<0.05 by Student t-test. (F) pAlu does not induce RPE degeneration in Myd88−/− heterozygous (het) mice. (G) Western blot of Alu RNA-induced IRAK1 and IRAK4 phosphorylation in human RPE cells. Image representative of 3 experiments. (H) pAlu reduces cell viability of WT but not Myd88−/− mouse RPE cells. (I) Loss of human RPE cell viability induced by pAlu is rescued by MyD88i. (J) AAV1-BEST1-Cre, but not AAV1-BEST1-GFP, protected Myd88−/− mice from pAlu-induced RPE degeneration. (K) pAlu induces IL-18 secretion from human RPE cells measured by ELISA. IL-17 secretion is barely detectable. n=3. *p<0.05 by Student t-test. (L) Recombinant IL-18 induces RPE degeneration in WT but not Myd88−/− mice. (M and N) pAlu-induced RPE degeneration in WT mice is rescued by IL-18 neutralizing antibody (N) but not by IL-16 neutralizing antibody (M). Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row. Degeneration outlined by blue arrowheads. Scale bars, 20 μm (A-C,F,L-N). n=3; *p<0.05 by Student t-test. Data are represented as mean±SEM (D,E,H,I,K). See also FIG. 9.

FIG. 3. Alu RNA induces RPE degeneration via NLRP3 inflammasome (A) Western blot of Caspase-1 activation (p20 subunit) by Alu RNA in human RPE cells. (B) Western blot of Alu-induced IL-18 maturation in RPE cell lysates in wild-type mice impaired by Caspase-1 peptide inhibitor. (C) Caspase-1 peptide inhibitor protects WT mice from pAlu-induced RPE degeneration. (D and E) pAlu does not induce RPE degeneration in Casp1−/− mice or from cytoxicity in Casp1−/− mouse RPE cells. (F) Alu RNA and LPS+ATP induce formation of PYCARD clusters in human RPE cells transfected with GFP-PYCARD. (G and H) pAlu does not induce RPE degeneration in Nlrp3−/− (G) or Pycard−/− (H) mice. (I) Nlrp3−/− and Pycard−/− mouse RPE cells are protected against pAlu-induced loss of cell viability. (J) siRNAs targeting NLRP3 or PYCARD rescued human RPE cells from pAlu-induced cytoxicity, compared to control siRNA. n=3-4. *p<0.05 by Student t-test (A,B,E,F,L). Images representative of 3 experiments. Densitometry values normalized to Vinculin are shown in parentheses (A,B).

FIG. 4. Alu RNA induces mitochondrial ROS production and NLRP3 priming (A) pAlu induces NLRP3 and IL18 mRNAs in WT and Myd88−/− mouse RPE cells. (B) pAlu induces generation of reactive oxygen species (ROS) in human RPE cells as monitored with the fluorescent probe H2DCFDA (A.U, arbitrary units). (C) DPI blocks pAlu-induced NLRP3 and IL18 mRNAs in human RPE cells. (D) DPI protects WT mice from pAlu-induced RPE degeneration. (E) pAlu induces generation of mitochondrial reactive oxygen species in human RPE cells as detected by the fluorescence of MitoSOX Red (green pseudocolor), colocalized with respiring mitochondria labeled by Mitotracker Deep Red (red). (F) PMA, but not pAlu, induces phagosomal ROS generation, as assessed by fluorescent Fc OXYBURST Green assay in human RPE cells. (A.U, arbitrary units). (G) MitoTempo and MitoQ, but not vehicle or dTPP, prevents Alu RNA-induced RPE degeneration in WT mice. (H) NADPH oxidase inhibitor gp91ds-tat or a scrambled peptide do not prevent Alu RNA-induced RPE degeneration in WT mice. (I) Alu RNA induces RPE degeneration mice deficient in Cybb (which encodes the gp91dhox subunit of NADPH oxidase). (J and K) siRNAs targeting MyDSS (siMyDSS) and NLRP3 (siNLRP3) rescued human RPE mROS visualized with MitoSox Red dye and cell nuclei with Hoechst stain. n=3-4, *p<0.05 by Student t-test (A-C, K), NS, not significant by Student t-test (F). Representative images shown. n=8-12. ZO-1 stained (red) flat mounts. Scale bars, 20 nm (D, E, G-I), n=3-4. Scale bar, 100 μm (J). See also FIG. 11.

FIG. 5. RPE degeneration does not occur via pyroptosis (A and B) Glycin inhibits human RPE cell death induced by LPS+ATP (A) but not by pAlu (B). (C) Recombinant IL-18 induces RPE degeneration in Casp1−/− mice. n=3-4 (A,B), *p<0.05 by Student t-test. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row. Degeneration outlined by blue arrowheads. Scale bars, 20 μm (C).

FIG. 6. Dicer1 loss induces cell death via inflammasome (A) Western blot of Alu RNA-induced Caspase-1 cleavage (p20) inhibited by Dicer1 overexpression in human RPE cells. (B and C) Dicer1 overexpression reduces Alu RNA-induced Caspase-1 activation in human RPE cells (measured by cleavage (B left panel, green) of Caspase-1 fluorescent substrate). Fluorescence quantification shown in right panel. (C) Western blot of increased Caspase-1 activation (p20 subunit) in RPE cell lysates of BEST1-Cre; Dicer1+/− mice compared to BEST1-Cre or Dicer1+/− mice. (D) Western blot of increased Caspase-1 activation (p20 subunit) and IL-18 maturation in RPE cell lysates of Dicer1+/− mice treated with AAV1-BEST1-Cre- (E and F) RPE degeneration induced by AAV1-BEST1-Cre in Dicer1+/− mice is rescued by peptide inhibitors of either Caspase-1 (E) or MyD88 (F). (G) MyD88 inhibitor rescues loss of human RPE cell viability induced by Dicer1 antisense (AS) treatment. (H) Dicer1 antisense (AS) treatment of human RPE cells reduces Dicer1 and increases IRAK1 and IRAK4 phosphorylation. (I) MyD88 inhibitor rescues loss of cell viability in Dicer1+/− mouse RPE cells treated with adenoviral vector coding for Cre recombinase (Ad-Cre). (J) Ad-Cre induced global miRNA expression deficits in Dicer1+/− mouse RPE cells compared to Ad-Null.
No significant difference in miRNA abundance between MyD88 inhibitor and control peptide-treated Dicer1 depleted cells. n=5 (A,B,F-H). Denstometry values normalized to Vinculin are shown in parentheses (A,C). Degeneration outlined by blue arrowheads. n=8 (E,F). *p<0.05 by Student t-test (G,I). Images representative of 3 experiments (A,B,C,D,I). See also FIG. 12. FIG. 7. NLRP3 Inflammasome and MyD88 activation in human GA (A) NLRP3 and IL18 abundance was significantly elevated in macular GA RPE (n=13) compared to normal age-matched controls (n=12). *p<0.05 by Mann-Whitney U-test. There was no significant difference between groups (p=0.32 by Mann-Whitney U-test) in IL18 abundance. (B-D) Increased immunolocalization of NLRP3 (B), PYCARD (C) and Caspase-1 (D) in macular GA RPE compared to age-matched normal controls. Scale bar, 20 μm. (E) Western blots of macular RPE lysates from individual human donor eyes show that abundance of NLRP3, PYCARD, and phosphorylated IRAK1/4, normalized to the levels of the housekeeping protein Vinculin, is reduced in geographic atrophy (GA) compared to age-matched normal controls. Data are represented as mean±SEM (A). Representative images shown. n=6 (B-E). See also FIG. 13. FIG. 8. Alu RNA does not activate several RNA sensors. (A and B) p7SL (a 7SL expression vector) (A) and in vitro synthesized 7SL RNA (B) do not induce RPE degeneration in wild-type mice. (C) RPE degeneration induced by subretinal injection of pAlu in wild-type mice is not blocked by a TLR4 antagonist. (D-E) Mice deficient in Mda5 (D) or Prkr (E) are susceptible to pAlu-induced RPE degeneration. (F) Dephosphorylated (Dep) Alu RNA induces RPE degeneration in wild-type mice just as well as Alu RNA. (G) Mice deficient in Mavs are susceptible to pAlu-induced RPE degeneration. pNull does not induce RPE degeneration in any strain of mice. Degeneration outlined by blue arrowheads. Fundus photographs, top rows; ZO-1 stained (red) RPE flat mounts, bottom rows. n=8 (A-G). (H) A schematic of the innate immune pathways that are not activated by Alu RNA.

FIG. 9. Alu RNA induces RPE degeneration via MyD88, not TRIF or IFNγ, (A) Subretinal administration of pAlu induces RPE degeneration in Ticolor1−/− mice. (B) Alu RNA does not induce RPE degeneration in Myd88−/− mice. (C) Subretinal administration of a different Alu expression plasmid (pAlu2) also induces RPE degeneration in wild-type but not Myd88−/− mice. (D) Alu RNA does not induce RPE degeneration in Myd88−/− heterozygous (het) mice. (E) MyD88 inhibitory peptide reduces Alu RNA-induced phosphorylation of IRAK1/4, normalized to Vinculin expression. (F) Subretinal injection of AAV1-BEST1-Cre, but not AAV1-BEST1-GFP, protects Myd88−/− mice from Alu RNA-induced RPE degeneration. (G) pAlu and Alu RNA induces RPE degeneration in wild-type mice receiving Myd88−/− bone marrow (Myd88−/−-wild-type) but did not do so in Myd88−/− mice receiving wild-type or Myd88−/− bone marrow (wild-type-Myd88−/−). (H-K) Subretinal administration of pAlu induces RPE degeneration in InIfg−/− (H), InIfgr1−/− (I), and Ifn1r1−/− mice (J) but not in Il18r1−/− mice (K). pNull administration does not induce RPE degeneration in any strain of mice. Degeneration outlined by blue arrowheads. Fundus photographs, top rows; ZO-1 stained (red) RPE flat mounts, bottom rows. n=8 (A-D,F-K).

FIG. 10. Alu RNA induces RPE degeneration via NLRP3 inflammasome activation, (A) Alu RNA or LPS+AIP induce activation of Caspase-1 in human RPE cells as assessed by increased cleavage of Caspase1® (green, left panel), a fluorescent-linked peptide substrate as compared to mock treatment. Fluorescence quantification shown in right panel. (B) Western blot of Alu RNA-induced Caspase-1 activation (p20 subunit) in THP-1 and HeLa cells, normalized to Vinculin expression. (C) Caspase-1 inhibitor peptide blocks Alu RNA-induced substrate cleavage in human RPE cells. n=3. (D) Subretinal injection of Alu RNA does not induce RPE degeneration in Casp1−/− mice. (E) Alu RNA or LPS+AIP induce the appearance of a brightly fluorescent cluster of GFP-PYCARD visible in the cytoplasm of human RPE cells. Arrow in insets shown in higher magnification. Images representative of 3 experiments. (F and G) Subretinal injection of Alu RNA does not induce RPE degeneration in Nlrp3−/− (F) or Pycard−/− (G) mice. (H) The abundance of NLRP3 in HEK293 cells transfected with an NLRP3 expression vector and of PYCARD in human RPE cells is reduced by transfection of siRNAs targeting these genes, compared to control (Ctrl) siRNAs. n=3, the relative ratio compared to Ctrl siRNAs by Student t-test. (I) Alu RNA-induced Caspase-1 activation (p20 subunit) in human RPE cells is unaffected by MyD88 inhibitory peptide, normalized to Vinculin expression. (J) MyD88 inhibitory peptide does not reduce Alu RNA-induced cleavage activity of Caspase-1 in human RPE cells (top panel). Fluorescence quantification (bottom panel). (K) Caspase-1 activation (p20 subunit) in RPE cell lysates of wild-type mice treated with subretinal pAlu administration is unimpaired by intravitreal administration of anti-IL-18 neutralizing antibodies. (L) Alu RNA-induced phosphorylation of IRAK1/4 is reduced by Caspase-1 inhibitory peptide in human RPE cells, normalized to Vinculin expression. Vehicle control injections also do not damage the RPE. Fundus photographs, top rows; ZO-1 stained (red) RPE flat mounts, bottom rows. n=8 (D,F,G). Images representative of 3 experiments (A,B,J-L).

FIG. 11. NLRP3 does not physically interact with Alu RNA, and VDAC knockdown by siRNA. (A) RNA-binding protein immunoprecipitation (RIP) assay in human RPE cells transfected with pAlu and pNLPR3-FLAG. Immunoprecipitation of protein-RNA complexes with antibodies against NLRP3 or FLAG did not reveal interaction between NLRP3 and Alu RNA. RNA isolated from an equal amount of cell lysate (not subjected to IP) was used as input for Alu PCR. Relative abundance of Alu RNA in the immunoprecipitate, assessed by real-time RT-PCR using Alu-specific primers, was normalized to levels obtained with control IgG immunoprecipitation. N=3. (B) The abundance of VCAD1, VCAD2, and VCAD3 mRNAs in human RPE cells is reduced by transfection of siRNAs targeting these genes compared to control (targeting luc) siRNA. N=3, *p<0.05 compared to Control siRNA by Student t-test. FIG. 12. DICER1 is a negative regulator of Caspase-1 activation by Alu RNA, (A) Knockdown of DICER1 by antisense oligonucleotides (AS) in human RPE cells increases cleavage activity of Caspase-1, as monitored by Caspase1®, a fluorescent (green in overlay) reporter of substrate cleavage compared to control AS treatment. (B) Inhibition of Alu RNA by AS treatment reduces Caspase1® fluorescence in human RPE cells treated with DICER1 AS. Mean values of Caspase1® fluorescence shown in parentheses. Images representative of 3 experiments.

FIG. 13. Schematic representation of proposed model of NLRP3 inflammasome activation by DICER1 deficient-induced Alu RNA that leads to RPE degeneration and geographic atrophy. Alu RNA induces priming of NLRP3 and IL18 mRNAs via generation of reactive oxygen species (ROS). Activation of the NLRP3 inflammasome triggers cleavage of pro-IL18 by activated Caspase-1 to mature
IL-18. IL-18 signals via MyD88 to phosphorylate IRAK1 and IRAK4, which leads to RPE cell death.

**FIG. 14.** Intravitreous administration of Caspase-8 inhibitor protects wild-type mice from pAlu-induced RPE degeneration. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

**FIG. 15.** Caspase-8 inhibitor protects human RPE cells from Alu induced cytotoxicity. Caspase-8 inhibitory peptide Z-IETD-FMK (100 µM) but not the control peptide Z-FA-FMK (100 µM) protects human RPE cells from Alu RNA-induced cytotoxicity.

**FIG. 16.** Caspase-8 inhibitor protects human RPE cells from pAlu-induced cytotoxicity. Caspase-8 inhibitory peptide Z-IETD-FMK (100 µM) but not the control peptide Z-FA-FMK (100 µM) protects human RPE cells from pAlu-induced cytotoxicity.

**FIG. 17.** IL-18 induced caspase-8 activation. Subretinal injection of IL-18 in wild-type mice induced activation of caspase-8, as monitored by fluorometric plate assay.

**FIG. 18.** pAlu does not induce RPE degeneration in CD95-/− mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

**FIG. 19.** Alu RNA does not induce RPE degeneration in CD95-/− mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

**FIG. 20.** Recombinant IL-18 does not induce RPE degeneration in CD95-/− mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

**FIG. 21.** pAlu does not induce RPE degeneration in Faslgr mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

**FIG. 22.** Alu RNA does not induce RPE degeneration in Faslgr mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

**FIG. 23.** Recombinant IL-18 does not induce RPE degeneration in Faslgr mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

**FIG. 24.** Alu RNA does not induce RPE degeneration in NIKb1-/- mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

**FIG. 25.** Alu RNA or vehicle (PBS) was injected into the subretinal space of fellow eyes of a wild-type mouse. 2-days later, DyeLight782-VAD-FMK3 was injected into the vitreous humor of both eyes. From baseline (0 hours) to 24 hours thereafter, photographs of the fundus (retina) were taken through the ICG filter of a Topcon 50IX camera. In the IL-18-injected eye, white fluorescent areas corresponding to bioactive caspase generation were observed in the area of IL-18 injection. No such widespread areas were observed in the vehicle-injected eye.

**FIG. 26.** Recombinant IL-18 or vehicle (PBS) was injected into the subretinal space of fellow eyes of a wild-type mouse. 2-days later, DyeLight782-VAD-FMK3 was injected into the vitreous humor of both eyes. From baseline (0 hours) to 24 hours thereafter, photographs of the fundus (retina) were taken through the ICG filter of a Topcon 50IX camera. In the IL-18-injected eye, white fluorescent areas corresponding to bioactive caspase generation were observed in the area of IL-18 injection. No such widespread areas were observed in the vehicle-injected eye.

**FIG. 27.** Recombinant IL-18 or vehicle (PBS) was injected into the subretinal space of fellow eyes of a wild-type mouse. 2-days later, DyeLight782-VAD-FMK3 was injected into the vitreous humor of both eyes. From baseline (0 hours) to 24 hours thereafter, photographs of the fundus (retina) were taken through the ICG filter of a Topcon 50IX camera. In the IL-18-injected eye, white fluorescent areas corresponding to bioactive caspase generation were observed in the area of IL-18 injection. No such widespread areas were observed in the vehicle-injected eye.

**FIG. 28.** Representative images show that subretinally injected Alu RNA (1 µg)-induced RPE degeneration is blocked by intravitreous administration of the MyD88 peptide inhibitor DRQIKWFQRMRKKWKRVD- LPGTCVWSIASE (2 µg). Top panels show color fundus photographs. Bottom panels show retinal flat mount preparations stained with an anti-ZO1 antibody (red). Alu RNA-induced RPE degeneration (left panels) is evidenced by degeneration seen on color photos (top left) and dysmorphic appearing RPE cells (bottom left). Treatment with the MyD88 peptide inhibitor prevents those degenerative changes and preserves normal RPE anatomy.

**BRIEF DESCRIPTION OF THE SEQUENCE LISTING**

SEQ ID NO: 1. IMG-2005-1 peptide sequence: DRQIKWFQRMRKKWKRVDLPGTCVWSIASE, wherein the last 7 amino acids are required for inhibition of MyD88 homodimerization, while the preceding amino acid sequence is an Antennapedia cell permeation sequence that enables the inhibitory peptide to enter the cell, so that it can block MyD88.

SEQ ID NO: 2. Control peptide sequence: DRQIKWFQRMRKKWKK

SEQ ID NO: 3. MyD88 siRNA #1 sense: 5'-GAGAAGGCUCUUAACAGGUCUdTdT-3'

SEQ ID NO: 4. MyD88 siRNA #1 antisense: 5'-ACCUGUAAGGCUUCUCUGGAdTdT-3'

SEQ ID NO: 5. MyD88 siRNA #2 sense: 5'-CAGAGCAAGGAAUGUGAdTdT-3'

SEQ ID NO: 6. MyD88 siRNA #2 antisense: 5'-UCACAUUCCUUCUGCUCCUAdTdT-3'

SEQ ID NO: 7. NLRP3 siRNA—5'-GUUUGACUAUCUGUCUAdTdT-3'

SEQ ID NO: 8. NLRP3 siRNA—5'-GGUCAACACUAUCUCUGUGA-3'

SEQ ID NO: 9. NLRP3 siRNA—5'-UGCAAGAACUCAGCACAAA-3'

SEQ ID NO: 10. NLRP3 siRNA—5'-GAAGUGGGGUCAGUAAAU-3'

SEQ ID NO: 11. NLRP3 siRNA—5'-GCAAGACCAAGCAGUGUGA-3'

SEQ ID NO: 12. PCARD siRNA—5'-GGCUCUCUCUGUUCUUCAdTdT-3'

SEQ ID NO: 13. PCARD siRNA—5'-GGCUCUCUCUGUUCUUCAdTdT-3'

SEQ ID NO: 14. PCARD siRNA—5'-GCCUCUCUCUGUUCUUCAdTdT-3'.

SEQ ID NO: 15: siRNA of the human Pyrin coding sequence: GCTGGAGCAGGTGTACTACTTC.

SEQ ID NO: 16: siRNA of the human NLRP3 coding sequence: CAGGTTTGACTATCTGTTCT.

SEQ ID NO: 17: siRNA of the human PYCARD coding sequence: GCTGGAGCAGGTGTACTACTTC.

SEQ ID NO: 18: Oligonucleotide primer for human MB, forward 5'-TTAAAGCCCGGCTTGACACA-3'

SEQ ID NO: 19: Oligonucleotide primer for human MB, reverse 5'-GCCAATGACAGGTTTTCTTAG-3').
The presently-disclosed subject matter includes methods for identifying MyD88 inhibitors, and methods and compositions for inhibiting MyD88 and uses thereof. The presently-disclosed subject matter includes methods for identifying inflammasome inhibitors, and methods and compositions for inhibiting an inflammasome and uses thereof. The presently-disclosed subject matter includes methods for identifying MyD88 inhibitors, and methods and compositions for inhibiting an inflammasome and uses thereof. Components of an inflammasome include, for example, NLRP3, PYCARD, and caspase-1.

In some embodiments of the method, the cell is selected from an RPE cell, a retinal photoreceptor cell, or a choroidal cell. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having a condition of interest. In some embodiments, the cell is an RPE cell. In some embodiments, the cell is the cell of a subject. In some embodiments, the cell is the cell of a subject having, suspected of having, or at risk of having a condition of interest.

DESCRIPTION OF EXEMPLARY EMBODIMENTS

The presently-disclosed subject matter includes methods for identifying MyD88 inhibitors, and methods and compositions for inhibiting MyD88 and uses thereof. The presently-disclosed subject matter includes methods for identifying inflammasome inhibitors, and methods and compositions for inhibiting an inflammasome and uses thereof. The presently-disclosed subject matter includes methods for identifying MyD88 inhibitors, and methods and compositions for inhibiting an inflammasome and uses thereof. Components of an inflammasome include, for example, NLRP3, PYCARD, and caspase-1.

The presently-disclosed subject matter includes methods for identifying inflammasome inhibitors, and methods and compositions for inhibiting an inflammasome and uses thereof. The presently-disclosed subject matter includes methods for identifying MyD88 inhibitors, and methods and compositions for inhibiting an inflammasome and uses thereof. Components of an inflammasome include, for example, NLRP3, PYCARD, and caspase-1.
atrophy. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having geographic atrophy and the cell is an RPE cell. In some embodiments, a subject having age-related macular degeneration can be treated using methods and compositions as disclosed herein.

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 inhibiting one or more of an inflammasome, MyD88, IL-18, VDAC1, VDAC2, NLRP3, PYCARD, caspase-1, caspase-8, and NFXB of a cell includes administering an inhibitor to the cell, or to a subject wherein this cell is the cell of a subject. Such inhibitors can be administered, for example, by intraocular injection (e.g., localized interocular therapy); intravitreal injection; sub-retinal injection; episcleral administration; sub-Tenon’s injection; retrobulbar injection; peribulbar injection; transscleral administration; topical administration, e.g., topical eye drop application; suprachoroidal administration; release from a sustained release delivery device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

As used herein the term “inhibit” or “inhibiting” refers to suppressing, reducing, decreasing, or substantially eliminating the biological activity of a polypeptide, such as MyD88, IL-18, VDAC1, VDAC2, caspase-8, NFXB, or a polypeptide of an inflammasome (e.g., NLRP3, PYCARD, caspase-1).

As used herein with reference to a polypeptide being inhibited, “of a cell” refers to a polypeptide that is inside the cell (inside the cell membrane), on the cell (in the cell membrane, presented on the cell membrane, otherwise on the cell), or outside of a cell, but insofar as the polypeptide is outside of the cell, it is in the extracellular milieu such that one of ordinary skill in the art would recognize the polypeptide as being associated with the cell. For example, VDAC1, VDAC2, caspase-8, NFXB, or a polypeptide of an inflammasome (e.g., NLRP3, PYCARD, caspase-1 of a cell could be in the cell. For another example MyD88 could be in the cell or on the cell. For yet another example, IL-18 could be outside the cell because it is secreted, but it would be recognized by one or ordinary skill in the art as being associated with the cell.

As will be understood by those skilled in the art upon studying the application, inhibition of an inflammasome, MyD88, IL-18, VDAC1, VDAC2, caspase-1, caspase-8, and NFXB of a cell can be achieved in a number of manners. In some embodiments the inhibition can be achieved by affecting the transcription or translation of the polypeptide, by degrading the polypeptide, by scavenging the polypeptide, or otherwise impacting the biological activity of the polypeptide. Inhibition comprises administering an inhibitor. An inhibitor is a compound that affects such inhibition of the biological activity of a polypeptide. Such compounds can be, for example, a polypeptide (including oligonucleotide, and including a polypeptide that binds to the polypeptide-of-interest to affect inhibition), a small molecule (including a small chemical compound), a compound for RNA interference (including siRNA, miRNA, shRNA), an antibody (e.g., a neutralizing antibody against polypeptide of interest, an antibody that blocks polypeptide of interest from binding to a receptor), an aptamer, a dominant negative plasmid or vector, or a virus-encoded inflammasome.

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 or carboxy-terminus of the reference polypeptide, from internal portions of the reference polypeptide, 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.

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 methods and compositions of the presently-disclosed subject matter can be used in a subject having, suspected of having, or at risk of having a condition of interest. In some embodiments, methods and compositions of the presently-disclosed subject matter can be used for treating a condition of interest. Examples of conditions of interest include, but are not limited to: Geographic atrophy (Kaneko, Dridi et al. 2011); Macular degeneration (Kaneko, Dridi et al. 2011); Keratitis (Guo, Gao et al. 2011); Gout (Chen, Shi et al. 2006); Acne vulgaris (Terhorst, Kalali et al. 2010); Crohn’s disease (Reuter and Pizarro 2004; Abreu, Fukata et al. 2005; Medvedev, Sabroe et al. 2006); Unerative colitis (Reuter and Pizarro 2004; Abreu, Fukata et al. 2005; Medvedev, Sabroe et al. 2006); irritable bowel disease/irritable bowel syndrome (McKernan, Nolan et al. 2009); Type 1 diabetes (Devaraj, Tobias et al. 2011; von Herrath, Filippi et al. 2011); Type 2 diabetes (Hutton, Soukhatcheva et al. 2010; Nogueira-Machado, Volpe et al. 2011); Insulin resistance (Ghanim, Mohanty et al. 2008; Tilich and Arora 2011); Obesity (Fresno, Alvarez et al. 2011); Hemolytic-Uremic Syndrome (Batsford, Duermueler et al. 2011); Polyoma virus infection (Batsford, Duermueller et al. 2011); Immune complex renal disease (Anders, Banas et al. 2004; Anders and Schlondorff 2007); Acute tubular injury (Anders, Banas et al. 2004; Anders and Schlondorff 2007); Lupus nephritis (Anders, Banas et al. 2004; Anders and Schlondorff 2007); Familial cold autoinflammatory syndrome (Mariathasan, Weiss et al. 2006; Meng, Zhang et al. 2009); Muckle-Wells syndrome and neonatal onset multisystem inflammatory disease (Mariathasan, Weiss et al. 2006; Meng, Zhang et al. 2009); Chronic infantile neurologic cutaneous and articular autoinflammatory diseases, Renal ischemia-perfusion injury (El-Achkar and Dagher 2006; Robson 2009); Glomerulonephritis (El-Achkar and Dagher 2006; Robson 2009); Cryoglobulinemia (Banas, Banas et al. 2008); Systemic vasculitides (Weyand, Ma-Krupa et al. 2005; Hurtado, Jeffs
as such, the terms treatment or treating include, but are 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.

in some embodiments, the methods and compositions of the presently-disclosed subject matter are useful for protecting the cell against Alu-RNA-induced degeneration. as such, in some embodiments, a method includes administering an inhibitor, wherein the cell is protected against Alu-RNA-induced degeneration.

inhibiting inflammasome

in some embodiments, the presently-disclosed subject matter includes a method of protecting a cell, comprising: inhibiting an inflammasome of the cell. the method of any one of the prior claims, wherein the inflammasome is selected from NLRP3 inflammasome, NLRP1 inflammasome, NLR4 inflammasome, AIM2 inflammasome, and IFI16 inflammasome. in some embodiments, the inflammasome is the NLRP3 inflammasome.

in some embodiments the inhibiting the inflammasome includes inhibiting a component of the inflammasome. in some embodiments the inflammasome components can include a polypeptide encoded by PYCARD. in some embodiments the inflammasome components can include a caspase.

in some embodiments, the inhibiting the inflammasome comprises administering an inflammasome inhibitor. the inflammasome inhibitor can be an inhibitor of a component of the inflammasome. in some embodiments, the inflammasome inhibitor is a component of the inflammasome.

as noted above, in some embodiments, inhibiting a polypeptide of interest to the presently-disclosed subject matter comprises administering an oligonucleotide or a small RNA molecule. such small RNA molecule can target, for example, NLRP3 and/or PYCARD. such nucleotides can target and degrade NLRP3 and/or PYCARD. in this regard, the presently-disclosed subject matter includes a method of targeting and degrading NLRP3 and/or PYCARD. wherein a first strand of the double-stranded RNA comprises a sequence as set forth in Table A, and includes about 14 to 25 nucleotides. as noted above, in some embodiments, inhibiting comprises administering an inflammasome inhibitor that is a dominant negative vector. in some embodiments, inhibiting comprises administering an inhibitor of Caspase-1. in some embodiments the inhibitor of Caspase-1 is a peptide inhibitor.

Examples of Inflammasome Inhibitors

ION channel inhibitors, for example, glybenclamide/glyburide (CAS Number: 10238-21-8) (Lamkanfi, et al., 2009).

IKK-α inhibitors, for example, BAY11-7082 (CAS Number: 195462-67-7; also known as (R)-3-(4-Methylphenylsulfonyl)-2-propenenitrile) (Juliana, et al., 2010).

Compounds similar to BAY11-7082, for example, other related vinyl sulfone compounds, as set forth in Lamkanfi, et al., 2009; Juliana, et al., 2010; deRivero Vaccari, et al., 2008; and Newman, et al., 2011, which are incorporated herein by this reference.
Examples of Inflammasome Inhibitors

Antibodies, for example, Anti-ASC and Anti-NALP1 and antibodies based on protein sequences selected from: ASC: ALR QTQ PYL VTD LEQ S; NALP1: MER SQS KEE SNT
EG-cys (deRivero Vaccari, et al., 2008); and Anti-NALP1 (Abcam, Cambridge, MA), anti-IL-1β (Cell Signaling Technology, Beverly, MA), anti-IL-18 (R & D Systems, Minneapolis, MN), anti-caspase-1 (Millipore, Billerica, MA), anti-caspase-1 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-caspase-11 (Alexis Biochemicals, San Diego, CA), anti-caspase-11 (Santa Cruz Biotechnology).

Direct inhibitors of Caspase-1 and/or NLRP3, for example, parthenolide (Juliana, et al., 2010).

Caspase-1 inhibitors, such as estrogen binding B-box protein (Munding et al., 2006); COP (Lee, et al., 2001), ICEBERG (Humke, et al., 2000), and Z-WEHD-FMK (R&D Systems).

Caspase 1 and/or 4 inhibitors, for example, Ac-YVAD-CHO (Ac-Tyr-Val-Ala-Asp-CHO) and Ac-YVAD-CMK (CAS Number, 178603-78-6; N-acetyl-L-tyrosyl-L-valyl-L-[N-[(1S)-1-(carboxymethyl)-3-chloro-2-oxo-propyl]-L-alaninamide) (Hilbi, et al., 1997).

Caspase-12 inhibitors (Saleh, et al., 2006).

Host-derived inhibitors of Caspase-1, for example, cellular PYRIN domain (PYD)-only proteins (POP) family: cPOPl and cPOP2 (Stehlik, et al., 2003; Dorfleutner, et al., 2007), serpin proteinase inhibitor 9 (PI-9) (Young, et al., 2000); BCL-2 and BCL-xL (Young, et al., 2000).

Inhibitors of Nlrp1b inflammasome, for example, auarofin (Newman, et al., 2011).

Virus expressed inhibitors of the inflammasome, for example, PYD homolog M13L-PYD, S013L (Benedict, et al., 2005; Dorfleutner, et al., 2007), SPI-2 homologs CrmA, Serp2, SPI-2, (Komiya, et al., 1994; Kettle, et al., 1997; Messud-Petit, et al., 1998), N1 (Stasakova, et al., 2005); Kaposi Sarcoma-associated Herpesvirus Orf63 (Gregory, et al., 2011).

Potassium chloride (KCl) (CAS Number, 7447-40-7 (Schorn, et al. 2011).

Cathepsin-B inhibitors, for example, CA-074 Me (L-3-trans-(Propylcarbamoyl)oxirane-2-Carbonyl)-L-Isoleucyl-L-Proline Methyl Ester (Li, et al., 2009). Cytochalasin D (Dostert, et al., 2008).

ROS inhibitors, for example, N-acetyl-L-cysteine (NAC), and (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (APDC) (Dostert, et al., 2008).

ASC-1 inhibitors, for example, cellular pyrin domain (PYD) superfamily proteins, also known as M013 (Rahman, et al., 2009).

NLRP3 inflammasome pan-caspase inhibitors, for example, Z-VAL-FMK (Dostert, et al., 2009).

Microtubules, for example, colchicine (CAS Number, 64-86-8) (Martinon, et al., 2006).

An isolated double-stranded RNA molecule that inhibits expression of NLRP3, and which can be conjugated to cholesterol or not, and at least one strand including the sequence: GUUUGACUAUCUGUUCUdTdT (SEQ ID NO, 7).

An isolated double-stranded RNA molecule that inhibits expression of NLRP3, at least one strand of which includes a sequence selected from: 5'-GGAUCAAACUACUCUGUGA-3' (SEQ ID NO, 10); 5'-GGCUGCUGGAUGCUCUGUACGGGAA-3' (SEQ ID NO, 13), and 5' -UUCCCGUACAGAGCAUCCAGCAGCC-3' (SEQ ID NO, 14) (Stealth siRNA oligos were designed and obtained with Lipofectamine 2000).
Further information regarding Caspase-1 inhibitors and probes can be found in Table B. Information found at the links set forth in Table B as of the filing date of this application is incorporated herein by this reference.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence Application</th>
<th>Link</th>
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</tr>
</thead>
<tbody>
<tr>
<td>GFVEVD</td>
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<td>Abz-GFVEVD-GVY(NO2)D</td>
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<tr>
<td>GVEVD</td>
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<td></td>
<td>fluoroscent</td>
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<td>Ac-YVAD-CHO</td>
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<tr>
<td></td>
<td>inhibitor</td>
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</tr>
<tr>
<td>WEDD</td>
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<td>Inhibitor</td>
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<tr>
<td></td>
<td>fluoroscent</td>
<td><a href="http://www.funakoshi.co.jp/data/datasheet/ORC/CPL1RE-5.pdf">http://www.funakoshi.co.jp/data/datasheet/ORC/CPL1RE-5.pdf</a></td>
<td>Caspalux</td>
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<tr>
<td>YEVD</td>
<td>fluorescent</td>
<td><a href="http://www.jbc.org/content/272/15/9677.long">http://www.jbc.org/content/272/15/9677.long</a></td>
<td>Ac-YVED-pNA</td>
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<tr>
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<td>Kinetic substrate</td>
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<td>T1.expansion.html</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Small molecule</th>
<th>Sequence Application</th>
<th>Link</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>VX-765</td>
<td>Inhibitor</td>
<td><a href="http://www.medkoo.com/Anticancer-trials/VX-765.htm">http://www.medkoo.com/Anticancer-trials/VX-765.htm</a></td>
<td>Vertex Pharmaceuticals, Reversible, clinical trials</td>
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<tr>
<td>CM-269</td>
<td>Reporter</td>
<td><a href="http://www.sciencedirect.com/science/article/pii/S1074552110003092#sec5.1">http://www.sciencedirect.com/science/article/pii/S1074552110003092#sec5.1</a></td>
<td>Luciferase based reporter</td>
</tr>
</tbody>
</table>

The presently-disclosed subject matter further includes compositions useful for inhibiting an inflammasome. Such compositions include an inhibitor. As noted above, such inhibitors can be, for example, a nucleotide, a polypeptide, a small (chemical) molecule, etc. In some embodiments, a composition can include an isolated RNA molecule.

The presently-disclosed subject matter includes isolated RNA molecules that inhibit expression of a component of inflammasome, e.g., NLRP3, caspase-1 and/or PYCARD. In some embodiments, a first strand of the double-stranded RNA comprises a sequence selected from the following, and including about 14 to 25 nucleotides: 5'-GUUUGAC-UAUCUGUUCdTdT-3' (SEQ ID NO: 7); 5'-GGAUC-CAACUACUGUGA-3' (SEQ ID NO: 8); 5'-UGCAGAUCACUGCGAAA-3' (SEQ ID NO: 9); 5'-GAAGUGGGGCUUCAGAUAAU-3' (SEQ ID NO: 10); 5'-GCAAGACCAAGACGUGUGA-3' (SEQ ID NO: 11); 5'-GAAGCUCUACUGUUCdTdT-3' (SEQ ID NO: 12); 5'-GGCUGCUGGAUGCUUCUGACGGGAA-3' (SEQ ID NO: 13); and 5'-UUCCCAGACAGCAGCAUGGAC-3' (SEQ ID NO: 14).

The presently-disclosed subject matter includes isolated RNA molecules that inhibit expression of an inflammasome component. In some embodiments, the RNA molecule comprises a sequence selected from the following:
The presently-disclosed subject matter further includes methods of screening candidate inhibitors to identify inflammasome inhibitors. In some embodiments, a method of identifying an inflammasome inhibitor makes use of a cultured cell wherein a cell based system is provided, which measures PYCARD aggregation, Caspase-1 cleavage, or cleavage/secretion of IL-1β or IL-18 in response to an activator of the inflammasome (e.g., Alu RNA, lipopolysaccharide-AIP).

In some embodiments, a screening method for inflammasome inhibitors includes stimulating cells (e.g., RPE cells) or a cell line (e.g., THP-1 or RAW macrophages) that has been transfected with a plasmid encoding a fluorescent sensor, such as CaspaLux fluorescence.

In some embodiments, a screening method for inflammasome inhibitors includes stimulating cells (e.g., RPE cells) or a cell line (e.g., THP-1 or RAW macrophages with a dominant negative or splice variant of MyD88, such as a MyD88 splice variant that lacks the death domain of MyD88 (Muzio et al. IRAK (Pelle) Family Member IRAK-2 and MyD88 as Proximal Mediators of IL-1 Signaling. Science 1997; 278:1612-1615). In such embodiments, the presently-disclosed subject matter includes a method of protecting a cell, comprising: inhibiting MyD88; and testing the candidate molecules for the degree of inhibition of Colometric signal.

Inhibiting MyD88

As noted above, in some embodiments, inhibiting MyD88 comprises administering an oligonucleotide or a small RNA molecule that inhibits expression of MyD88, wherein a first strand of the double-stranded RNA comprises a sequence set forth in Table C, and includes about 14 to 25 nucleotides. Examples of MyD88 inhibitors that can be used in accordance with the presently-disclosed subject matter include, but are not limited to those set forth in Table C. As such, embodiments of the presently-disclosed subject matter can include administering a MyD88 inhibitor set forth in Table C.

TABLE C

<table>
<thead>
<tr>
<th>Examples of MyD88 Inhibitors</th>
</tr>
</thead>
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<tr>
<td>An isolated double-stranded RNA molecule that inhibits expression of MyD88, at least one strand of which is about 14 to 25 nucleotides and includes a sequence selected from: 5’-GAGAAGCCUUUACAGGUdTdT-3’ (SEQ ID NO: 3); 5’-ACCUGUAAAGGCUUCUdTdT-3’ (SEQ ID NO: 4); 5’-CAAGGGAGACGUGUGAAGUdTdT-3’ (SEQ ID NO: 5); 5’-UCACUCUUCUGUCUGUGAAGUdTdT-3’ (SEQ ID NO: 6); and 5’-UAUUUCUUAANGGAGUdTdT-3’ (SEQ ID NO: 56).</td>
</tr>
<tr>
<td>A homodimerization inhibitor, such as Pepinh-MYD (Invitrogen).</td>
</tr>
<tr>
<td>A dominant negative or splice variant of MyD88, such as a MyD88 splice variant that lacks exon 2 (also known as the “intermediate domain” (e.g., having sequences set for at accession numbers NM_001172566.1 and NM_001172568.1), or other splice variants of MyD88 (e.g., having sequences set for at accession numbers NM_002468.4 and NM_001172569.1).</td>
</tr>
</tbody>
</table>
weight mimic of the Toll/IL-1 receptor/resistance domain inhibits IL-1 receptor-mediated responses.” PNAS 2003; 100: 7971-7976; or (2) S2285 as described in Carminati, P., Gallo, G., Ruggiero, V., Sassano, M., Mastroianni, D. “MyD88 homodimerization inhibitors” Patent No. WO2006067091 and characterized in Loiarro et al. “Inhibition of MyD88 dimerization and recruitment of IRAK1 and IRAK4 by a novel peptidomimetic compound.” Journal of Leukocyte Biology. 2007; 82:801-810; or (3) 4-[(E)-2-[1-hexylpyridin-1-ium-2-yeethenyl]-N,N-dimethylaniline iodide, also known as 4-[(E)-2-[1-hexylpyridin-6-yl]ethenyl]-N,N-dimethylaniline iodide, also known as Chemical Structure CID 5713657 in PubChem which blocks MyD88 interactions, or (4) the compounds referred to as 50-F12 and 26-J10 in Lee et al. “Application of β-Lactamase Enzyme Complementation to the High-Throughput Screening of Toll-Like Receptor Signaling Inhibitors.” Molecular Pharmacology 2007; 72:868-875), or a natural product (malyn-gamide F acetate as described in Villa et al. “Selective MyD88-dependent pathway inhibition by the cyanobacterial natural product malyn-gamide F acetate.” European Journal of Pharmacology 2010; 629:140-146), or a DNA or RNA aptamer generated by SELEX or other screening technology that binds or blocks MyD88.

The presently-disclosed subject matter further includes compositions useful for inhibiting MyD88. Such compositions include an inhibitor. As noted above, such inhibitors can be, for example, a nucleotide, a polypeptide, a small (chemical) molecule, etc. In some embodiments, a composition can include an isolated RNA molecule.

The presently-disclosed subject matter includes isolated RNA molecules that inhibit expression of MyD88. In some embodiments, a first strand of a double-stranded RNA comprises a sequence selected from the following, and including about 14 to 25 nucleotides: 5'-GAGAGCGCUUACAGGGCGUdTdT-3' (SEQ ID NO: 4); 5'-ACCUCGUAAAGCGUUCUCUGdTdT-3' (SEQ ID NO: 5); and 5'-UCACAUUUCUGCUUGCUGdTdT-3' (SEQ ID NO: 6).

In some embodiments, the polypeptide molecule comprises a sequence selected from the following: DQIKWFQNRKMKKWKRDVLPGT (SEQ ID NO: 1), including about 29 to 100 amino acids. In some embodiments, the polypeptide molecule comprises a sequence selected from the following: RDVLPGT (SEQ ID NO: 54) and RDVPAGG (SEQ ID NO: 55).

In some embodiments, a method of identifying a MyD88 inhibitor makes use of a cultured cell wherein MyD88 is upregulated. Candidate compounds can be screened using the cultured cell to determine efficacy in modulating MyD88. 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, and including siRNAs, antibodies, aptamers, and dominant negative plasmids or vectors.

In some embodiments, the screening method includes providing a cell in culture wherein MyD88 is upregulated; and contacting a candidate compound with the cell. The method can further include identifying a change in MyD88. For example, a measurable change in MyD88 levels can be indicative of efficacy associated with the candidate compound. In some embodiments, wherein the change in the MyD88 is a measurable decrease in MyD88, the change is an indication that the candidate compound is a MyD88 inhibitor. Such MyD88 inhibitors can have utility for therapeutic applications as disclosed herein.

In some embodiments, the MyD88 can be upregulated using Alu RNA or lipopolysaccharide (LPS), for example, by stimulating cells (macrophages or RPE cells) with Alu RNA or LPS. In some embodiments, the MyD88 can be upregulated using CpG nucleotides, for example, by stimulating cells (macrophages or RPE cells) with synthetic oligonucleotides containing unmethylated CpG dinucleotides, such as 5'-tcg ttg ttt ggt ggt gg-3' or 5'-ggG GGA CGA TCG TCG ggG ggG-3'. In some embodiments, the MyD88 can be upregulated using interleukin-1 beta or interleukin 18, for example, by stimulating cells (macrophages or RPE cells) with recombinant forms of interleukin-1 beta or interleukin 18.

In some embodiments of the method for identifying a MyD88 inhibitor, a change in the MyD88 can be monitored by measuring cell viability, measuring the expression of a gene known to be induced by MyD88 signaling (e.g., Cox-2, Socs3, TNF-alpha), or using other criteria that would be recognized by one of ordinary skill in the art, using methods known to one of ordinary skill in the art. In some embodiments, the cultured cell is an RPE cell. In some embodiments, the cell is a retinal photoreceptor cell. In some embodiments, the cell is a choroidal cell.

In some embodiments of a method for identifying a MyD88 inhibitor includes providing a cultured cell wherein MyD88 is upregulated or undergoes oligomerization or induces phosphorylation of IRAK1 or of IRAK4; and contacting the cell with a candidate compound; and determining whether the candidate compound results in a change in the MyD88 levels, or a change in the abundance of dimerized or oligomerized MyD88, or a change in the abundance of phosphorylated IRAK1 or of phosphorylated IRAK4. In some embodiments, the MyD88 is upregulated by: Alu RNA, lipopolysacharide, CpG nucleotides, single-stranded RNA, interleukin-1 beta, or interleukin 18. In some embodiments, the MyD88 is monitored by measuring cell viability, or measuring the expression of a gene known to be induced by MyD88 signaling.

In some embodiments of the method for identifying a MyD88 inhibitor, the gene known to be induced by MyD88 signaling is selected from Cox-2, Socs3, and TNF-alpha.

In some embodiments of a screening method for MyD88 inhibitors, cells or cell lines can be stimulated with a known activator of MyD88, e.g., Alu RNA, or LPS. The RNA levels of genes such as Cox2, Socs3, or TNF-alpha can be measured using quantitative real-time RT-PCR. Candidate molecules can be tested for degree of inhibition of these gene transcripts.

In some embodiments of a screening method for MyD88 inhibitors, cells or cell lines can be stimulated with a known activator of MyD88, e.g., Alu RNA, or LPS. The abundance of dimerized or oligomerized MyD88 can be measured by Western blotting under non-reducing conditions using an anti-MyD88 antibody. The candidate molecule can be tested for degree of inhibition of MyD88 dimerization or oligomerization.

In some embodiments of a screening method for MyD88 inhibitors, cells or cell lines that have been transfected with plasmids coding for a fusion MyD88 protein tagged to fragments of YFP (yellow fluorescent protein) can be stimulated with a known activator of MyD88, e.g., Alu RNA, or LPS. The fluorescent signal can be measured using bimolecular fluorescence complementation techniques. The candidate molecule can be tested for degree of inhibition of fluorescent signal.
In some embodiments of a screening method for MyD88 inhibitors, cells or cell lines can be stimulated with a known activator of MyD88, e.g., Alu RNA, or LPS. The abundance of phosphorylated forms of IRAK1 or IRAK4 can be measured by Western blotting under reducing conditions using an anti-phosphoIRAK1 or anti-phosphoIRAK4 antibody. The candidate molecule can be tested for degree of inhibition of IRAK1 or IRAK4 phosphorylation.

Inhibiting IL-18

In some embodiments, the presently-disclosed subject matter includes a method of protecting a cell, comprising: inhibiting IL-18 of the cell. In some embodiments, the inhibiting IL-18 comprises administering an IL-18 inhibitor.

As noted above, in some embodiments, inhibiting a polypeptide of interest to the presently-disclosed subject matter comprises administering a binding protein or an antibody. Such antibodies can include a neutralizing antibody against IL-18, or an antibody that blocks IL-18 binding to the IL-18 receptor. In some embodiments, the IL-18 inhibitor can be an IL-18 binding protein (Novick, et al., 1999).

Examples of IL-18 inhibitors that can be used in accordance with the presently-disclosed subject matter include, but are not limited to those set forth in Table D. As such, embodiments of the presently-disclosed subject matter can include administering an IL-18 inhibitor set forth in Table D.

<table>
<thead>
<tr>
<th>Examples of IL-18 Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>An isolated double-stranded RNA molecule that inhibits expression of VDAC1, at least one strand of which is about 14 to 25 nucleotides and includes the sequence of: 5'-CGUACGUGCACGCAGCTG-3' (SEQ ID NO: 47).</td>
</tr>
<tr>
<td>An isolated double-stranded RNA molecule that inhibits expression of VDAC2, at least one strand of which is about 14 to 25 nucleotides and includes the sequence of: 5'-CCUGUAGUGUGUGUGUGUGUGUGUGUGUGUGUGUG-3' (SEQ ID NO: 48).</td>
</tr>
<tr>
<td>Cyclosporin A-blocks VDAC1</td>
</tr>
<tr>
<td>Superoxide dismutase 1-blocks VDAC1</td>
</tr>
<tr>
<td>4,4'-diisothiocyanatoetilene-2,2'-disulfonic acid (DIDS)-blocks VDAC1</td>
</tr>
<tr>
<td>Bcl-x(L) BH4(4-23)-blocks VDAC</td>
</tr>
<tr>
<td>TR019622-blocks VDAC</td>
</tr>
</tbody>
</table>

Inhibiting VDAC1 and/or VDAC2

In some embodiments, the presently-disclosed subject matter includes a method of protecting a cell, comprising: inhibiting VDAC1 and/or VDAC2 of the cell. In some embodiments, the inhibiting VDAC1 and/or VDAC2 comprises administering an VDAC1 and/or VDAC2 inhibitor.

As noted above, in some embodiments, inhibiting a polypeptide of interest to the presently-disclosed subject matter comprises administering an oligonucleotide or a small RNA molecule. Such small RNA molecule can target VDAC1 and/or VDAC2. Such nucleotides can target and degrade VDAC1 and/or VDAC2. In this regard, the presently-disclosed subject matter includes a isolated double-stranded RNA molecule that inhibits expression of VDAC1 and/or VDAC2, wherein a first strand of the double-stranded RNA comprises a sequence as set forth in Table E, and includes about 14 to 25 nucleotides. Examples of VDAC1 and/or VDAC2 inhibitors that can be used in accordance with the presently-disclosed subject matter include, but are not limited to those set forth in Table E. As such, embodiments of the presently-disclosed subject matter can include administering a VDAC1 and/or VDAC2 inhibitor set forth in Table E.
The presently-disclosed subject matter further includes compositions useful for inhibiting VDAC1 and/or VDAC2. Such compositions include an inhibitor. As noted above, such inhibitors can be, for example, a nucleotide, a polypeptide, a small (chemical) molecule, etc. In some embodiments, a composition can include an isolated RNA molecule.

The presently-disclosed subject matter includes isolated RNA molecules that inhibit expression of VDAC1 and/or VDAC2. In some embodiments, a first strand of the double-stranded RNA comprises a sequence selected from the following, and including about 14 to 25 nucleotides: 5’-CG-GAAUAGCAGCCAAGUdTdT-3’ (SEQ ID NO: 47) and 5’-CCCUGGAGUUGGAGGCUdTdT-3’ (SEQ ID NO: 48).

The presently-disclosed subject matter further includes methods of screening candidate inhibitors to identify VDAC1 and/or VDAC2 inhibitors. In some embodiments, cell or cell line-based methods are used.

Inhibiting Caspase-8

In some embodiments, the presently-disclosed subject matter includes a method of protecting a cell, comprising: inhibiting caspase-8 of the cell. In some embodiments, the inhibiting caspase-8 comprises administering a caspase-8 inhibitor.

Examples of caspase-8 inhibitors that can be used in accordance with the presently-disclosed subject matter include, but are not limited to those set forth in Table F. As such, embodiments of the presently-disclosed subject matter can include administering a caspase 8 inhibitor set forth in Table F.

<p>| TABLE F |</p>
<table>
<thead>
<tr>
<th>Examples of Caspase-8 Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-ETD-FMK (BD Biosciences)</td>
</tr>
<tr>
<td>Ac-Ala-Ala-Val-Ala-Leu-Leu-Pro-Ala-Ala-Leu-Leu-Leu-Ala-Leu-Leu-Ala-Pro-Ile-Glu-Thr-Asp-CHO (EMD Millipore)</td>
</tr>
<tr>
<td>Z-Ile-Glu(Ome)-Thr-Asp(Ome)-CH₂F (EMD Millipore)</td>
</tr>
</tbody>
</table>

In some embodiments, the presently-disclosed subject matter includes compositions useful for inhibiting caspase-8. Such compositions include an inhibitor. As noted above, such inhibitors can be, for example, a nucleotide, a polypeptide, a small (chemical) molecule, etc. In some embodiments, a composition can include an isolated RNA molecule.

The presently-disclosed subject matter further includes methods of screening candidate inhibitors to identify caspase-8 inhibitors. In some embodiments, cell or cell line-based methods are used.

Inhibiting NFκB

In some embodiments, the presently-disclosed subject matter includes a method of protecting a cell, comprising: inhibiting NFκB of the cell. In some embodiments, the inhibiting NFκB comprises administering a caspase-8 inhibitor.

Examples of NFκB inhibitors that can be used in accordance with the presently-disclosed subject matter include, but are not limited to those set forth in Table G. As such, embodiments of the presently-disclosed subject matter can include administering a NFκB inhibitor set forth in Table G.

<p>| TABLE G |</p>
<table>
<thead>
<tr>
<th>Examples of NFκB Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidants that have been shown to inhibit activation of NF-kB</td>
</tr>
<tr>
<td>α-Lipoic acid</td>
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<tr>
<td>α-tocopherol</td>
</tr>
<tr>
<td>Aged garlic extract (alllicin)</td>
</tr>
<tr>
<td>2-Methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)</td>
</tr>
<tr>
<td>N-acetylornithine dimers (from P. cinctae)</td>
</tr>
<tr>
<td>Allsupranol</td>
</tr>
<tr>
<td>Anetholethione</td>
</tr>
<tr>
<td>Apocynin</td>
</tr>
<tr>
<td>Apple juice/extracts</td>
</tr>
<tr>
<td>Aseroeeria p7F (5,6,7,5'-tetramethoxy 7,4'-hydroxyflavone)</td>
</tr>
<tr>
<td>Antaxanthin</td>
</tr>
<tr>
<td>Autumn olive extracts; olive leaf extracts</td>
</tr>
<tr>
<td>Avenanthramides (from oats)</td>
</tr>
<tr>
<td>Bamboo culm extract</td>
</tr>
<tr>
<td>Bendopidine</td>
</tr>
<tr>
<td>bas-exugenol</td>
</tr>
<tr>
<td>Bruguiera gymnorrhiza compounds</td>
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<tr>
<td>Butylated hydroxyanisole (BHA)</td>
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<tr>
<td>Cepharanthine</td>
</tr>
<tr>
<td>Caffeic Acid</td>
</tr>
<tr>
<td>(3,4-dihydroxycinnamic acid, CAFE)</td>
</tr>
<tr>
<td>Carnosol</td>
</tr>
<tr>
<td>beta-Carotene</td>
</tr>
<tr>
<td>Carvedilol</td>
</tr>
<tr>
<td>Catechol Derivatives</td>
</tr>
<tr>
<td>Centaurea L. (Asteraceae) extracts</td>
</tr>
<tr>
<td>Chalcone</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
</tr>
<tr>
<td>3-chloroanisyl-2-anino-1,3-selenazoles</td>
</tr>
<tr>
<td>Cholestin</td>
</tr>
<tr>
<td>Chroman-2-carboxylic acid N-substituted phenylamides</td>
</tr>
<tr>
<td>Cocoa polyphenols</td>
</tr>
<tr>
<td>Coffee extract (3-methyl-1,2-cyclopentanediane)</td>
</tr>
<tr>
<td>Curcussus pinnatifida polyphenols</td>
</tr>
</tbody>
</table>

Examples include administering a NFKB inhibitor set forth in Table G.
<table>
<thead>
<tr>
<th>Examples of NFKB Inhibitors</th>
<th>Any one or more of the following NFKB inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cucurmin (Diferuloylmethane); dimerethoxycurcumin; EPZ4 analog</td>
<td>Singh &amp; Aggarwal, 1995; Pue et al, 2008; Kasimkin et al, 2008</td>
</tr>
<tr>
<td>Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS)</td>
<td>Iwasaki et al, 2004; Liu et al, 2005</td>
</tr>
<tr>
<td>Dihydroxybutyrlactone lignans</td>
<td>Cho et al, 2002</td>
</tr>
<tr>
<td>Dihydroethylcarbamates (DDEC)</td>
<td>Schreck et al, 1992</td>
</tr>
<tr>
<td>Diferoxamine</td>
<td>Supperti et al, 1995; Schreck et al, 1992</td>
</tr>
<tr>
<td>Dihydricrassourenginol; isoucenginol; epoxysesoioiucenginol-2-methyl butyrate</td>
<td>Murakami et al, 1995; Tark et al, 2007; Ma et al, 2008</td>
</tr>
<tr>
<td>Dihydridric Acid</td>
<td>Suzuki et al, 1992, 1995</td>
</tr>
<tr>
<td>Dileupe + ferulic acid</td>
<td>Sonoki et al, 2003; Yang et al, 2005</td>
</tr>
<tr>
<td>Dimethylthiouracilates (DMTC)</td>
<td>Pyatt et al, 1998</td>
</tr>
<tr>
<td>Danethyliulfoxide (DMSO)</td>
<td>Kelly et al, 1994</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>Schreck et al, 1992</td>
</tr>
<tr>
<td>Dihydrolipoic Acid</td>
<td>Schreck et al, 1992</td>
</tr>
<tr>
<td>Edanancrone</td>
<td>Hirano et al, 1998</td>
</tr>
<tr>
<td>EPC-K1 (phosphodiester compound of vitamin E and vitamin C)</td>
<td></td>
</tr>
<tr>
<td>Epeddylololucid-3-gallate (EOCG; green tea polyphenols)</td>
<td></td>
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<tr>
<td>Ergothioneine</td>
<td></td>
</tr>
<tr>
<td>Ethyl Pyruvate (Glutathione depletion)</td>
<td></td>
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<tr>
<td>Ethylene Glycol Tetracetic acid (EGTA)</td>
<td></td>
</tr>
<tr>
<td>Exaplastin</td>
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<tr>
<td>Exercise</td>
<td></td>
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<tr>
<td>Fisetin</td>
<td></td>
</tr>
<tr>
<td>Flavonoids (Centegae; 2hoolharia diffusa root; xantheloma)</td>
<td></td>
</tr>
<tr>
<td>Epatherum arnottianum; genistein; Isosteviol</td>
<td></td>
</tr>
<tr>
<td>Eucalyptus</td>
<td></td>
</tr>
<tr>
<td>Flavonoids and tert-butyl hydroquinone)</td>
<td></td>
</tr>
<tr>
<td>Flavonoids (extra-virgin olive oil)</td>
<td></td>
</tr>
<tr>
<td>Folic acid</td>
<td></td>
</tr>
<tr>
<td>Ganoderma lucidum polysaccharides</td>
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</tr>
<tr>
<td>Gamma-glutamylcysteine</td>
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<tr>
<td>Glutathione</td>
<td></td>
</tr>
<tr>
<td>Ginkgo biloba extract</td>
<td></td>
</tr>
<tr>
<td>Glutamine in (GCS)</td>
<td></td>
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<tr>
<td>Guacamol (from extract of Garcinia indica fruit rind)</td>
<td></td>
</tr>
<tr>
<td>Hydroquinone</td>
<td></td>
</tr>
<tr>
<td>Ibayten</td>
<td></td>
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<tr>
<td>Isosteviol</td>
<td></td>
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<tr>
<td>Isosacchurin</td>
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<tr>
<td>Justicia gendarussa root extract</td>
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<tr>
<td>Kallistatin</td>
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<tr>
<td>Kargen-karyu extract</td>
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<td>Kaurone</td>
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<tr>
<td>Lactisilene</td>
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<td>Lecithin</td>
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<td>Lisoacarbol</td>
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<td>Licoberries</td>
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<tr>
<td>Lipid</td>
<td></td>
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<td>Lutein</td>
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<td>Magnolol</td>
<td></td>
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<tr>
<td>Maitel</td>
<td></td>
</tr>
<tr>
<td>Manganese superoxide dismutase (Mn-SOD)</td>
<td></td>
</tr>
<tr>
<td>Melatonin</td>
<td></td>
</tr>
<tr>
<td>21 (alpha, beta)-methylmelianidol</td>
<td></td>
</tr>
<tr>
<td>Mulberry anthocyanins</td>
<td></td>
</tr>
<tr>
<td>N-acetyl-L-cysteine (NAC)</td>
<td></td>
</tr>
<tr>
<td>Nacysefulny (NAL)</td>
<td></td>
</tr>
<tr>
<td>Norexydrogluassic acid (NGDA)</td>
<td></td>
</tr>
<tr>
<td>Ochladavine</td>
<td></td>
</tr>
<tr>
<td>Onion extract (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyranone)</td>
<td></td>
</tr>
<tr>
<td>Orthophenanthrolime</td>
<td></td>
</tr>
<tr>
<td>N-(3-oxo-dodecanoyl) homoserine lactone</td>
<td></td>
</tr>
<tr>
<td>Panaxadoline</td>
<td></td>
</tr>
<tr>
<td>Phenolic antioxidants (Hydroquinone and tert-butyl hydroquinone)</td>
<td></td>
</tr>
<tr>
<td>Olive oil phenols (extra-virgin olive oil)</td>
<td></td>
</tr>
<tr>
<td>Alkenylphenols from Piper obliquum</td>
<td></td>
</tr>
<tr>
<td>Aloe vera (commercially available)</td>
<td></td>
</tr>
<tr>
<td>Antioxidants (Hydroquinone (Vitamin E-like compound)</td>
<td></td>
</tr>
<tr>
<td>Ban et al, 2007; Ofta et al, 2008</td>
<td></td>
</tr>
<tr>
<td>Examples of NFKB Inhibitors</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td></td>
</tr>
<tr>
<td>any one or more of the following NFKB inhibitors</td>
<td></td>
</tr>
</tbody>
</table>

- alpha-phenyl-n-tert-butyl-nitron (PBN)
- Phyllanthus urinaria
- Phytopherol furadates (rice bran)
- Piper longum Linn. extract
- Pitafluvatia
- Pterostilbene
- Pyrrolinedibrocarbamate (PDTC)
- Quercetin
- Kotake et al, 1998; Lin et al, 2006
- Arbaut et al, 1998
- Chiaralongo et al, 2005; Shen et al, 2007
- Singh et al, 2007
- Tounai et al, 2007; Wang & Kitajima, 2007
- Heu et al, 2007
- Cichocki et al, 2008; Panet al, 2009
- Schreck et al, 1992
- Kotake et al, 1998; Lin et al, 2006
- Arbaut et al, 1998
- Cichocki et al, 2008; Panet al, 2009
- Schreck et al, 1992
- Kotake et al, 1998; Lin et al, 2006
- Arbaut et al, 1998
- Cichocki et al, 2008; Panet al, 2009
- Schreck et al, 1992
- Kotake et al, 1998; Lin et al, 2006
- Arbaut et al, 1998
- Cichocki et al, 2008; Panet al, 2009
- Schreck et al, 1992
- Kotake et al, 1998; Lin et al, 2006
- Arbaut et al, 1998
- Cichocki et al, 2008; Panet al, 2009
- Schreck et al, 1992
- Kotake et al, 1998; Lin et al, 2006
- Arbaut et al, 1998
- Cichocki et al, 2008; Panet al, 2009
- Schreck et al, 1992
- Kotake et al, 1998; Lin et al, 2006
- Arbaut et al, 1998
- Cichocki et al, 2008; Panet al, 2009
- Schreck et al, 1992
- Kotake et al, 1998; Lin et al, 2006
- Arbaut et al, 1998
- Cichocki et al, 2008; Panet al, 2009
- Schreck et al, 1992
- Kotake et al, 1998; Lin et al, 2006
- Arbaut et al, 1998
- Cichocki et al, 2008; Panet al, 2009
- Schreck et al, 1992

**Proteasome inhibitors**

- Peptide Aldehydes:
  - A.L.L.
  - (N-acetyl-leucinyl-leucinyl-norleucynyl, MGIH1)
  - L.L.M. (N-acetyl-leucinyl-leucinyl-norleucynyl)
  - Z.L.L.
  - (carbobenzoxy-leucinyl-leucinyl-norvalinal, MGIH15)
  - Z.L.L.
  - (carbobenzoxy-leucinyl-norleucynyl, MGIH32)
  - M0262
  - Lactacystine, beta-lactone
  - Boronic Acid Peptide
  - Dithiocarbamate complexes with metals
  - CEP18770
  - Ubiquitin Ligase Inhibitors
  - PS-341 (Bortezomib)
  - Salinosporamide A (1, NPL-0052)
  - Cyclosporin A
  - McCaffrey et al, 1994; Meyer et al, 1997; Wechsler et al, 1994
  - FK506 (Tacrolimus)
  - Deoxycoformycin
  - Doxorubicin
  - Doniflamin
  - PT-110
  - Protease inhibitors
  - APNE (N-acetyl-DL-phenylalanine-b-naphthylester)
  - BIEE (N-benzoyl-L-tyrosine-ethyllester)
  - DCIC (3,4-dichloroisocoumarin)
  - DEF (disopropyl fluoro phosphate)
  - TPCK (N-a-tosyl-L-phenylalaize chloromethyl ketone)


- Pujois et al, 2012
- Fang et al, 1998; Grisham et al, 1999
- Grisham et al, 1999; Jobin et al, 1998
- Cevol & Dvorak, 2007
- Fiva et al, 2007
- Yaun et al, 1997
- Adamci et al, 2004
- Machtret al, 2005; Ahn et al, 2007
- Frantz et al, 1994; Kunz et al, 1995; Marienfeld et al, 1997;
- Okamoto et al, 1994; Venkatakrishnan et al, 1995
- Topper et al, 1995
- Lebovog et al, 2005
- Momose et al, 2007
- Iguchi et al, 1995
- Rossi et al, 1998
- D'Acquisto et al, 1998
**Examples of NFκB Inhibitors**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Point of Inhibition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desfordinatine; diphenhydramine Histamine H1 receptor</td>
<td>Wα et al, 2004; Scadding, 2005;</td>
<td>Roumestani et al, 2008</td>
</tr>
<tr>
<td>BKAmin</td>
<td>LPS receptor agonists</td>
<td>Kobayashi, 1996;</td>
</tr>
<tr>
<td>Rosa Tyrosine kinase receptor</td>
<td>Suppresses TNF production</td>
<td>Kamiyama et al, 2007</td>
</tr>
<tr>
<td>TAK-242</td>
<td>TRAF4 intracellular domain</td>
<td>Lentsch et al, 2007</td>
</tr>
<tr>
<td>Sulindac, fluticacne propionate</td>
<td>beta2 agonists</td>
<td>Kawamata et al, 2008</td>
</tr>
<tr>
<td>CPU0213</td>
<td>Endothelin receptor antagonist</td>
<td>Biscar, 2005</td>
</tr>
<tr>
<td>Doxazosin</td>
<td>alpha1-adrenergic receptor antagonist</td>
<td>He et al, 2006</td>
</tr>
<tr>
<td>Eribin overexpression</td>
<td>NO2 inhibitor</td>
<td>Hai et al, 2007</td>
</tr>
<tr>
<td>Protein-bound polysaccharide from basidionycetes</td>
<td>LPS-CD14 interaction</td>
<td>McDonald et al, 2005</td>
</tr>
<tr>
<td>Anti-CD146 antibody AA98</td>
<td>upstream of IKK (TRAF2-NIK)</td>
<td>Asai et al, 2005</td>
</tr>
<tr>
<td>Calagnaline (fern derivative)</td>
<td>upstream of IKK</td>
<td>Bu et al, 2006</td>
</tr>
<tr>
<td>NS3/4A (HCV protease)</td>
<td>upstream of IKK (PKC)</td>
<td>Manna et al, 2003</td>
</tr>
<tr>
<td>geloli IG2 (product of myelination basic protein)</td>
<td>Tnf2 inhibition</td>
<td>Kamiyama et al, 2005</td>
</tr>
<tr>
<td>NPM-ALK oncogene protein</td>
<td>Tnf2 inhibition</td>
<td>Feng et al, 2004</td>
</tr>
<tr>
<td>NS5A (Hepatitis C virus)</td>
<td>F3 Kinase inhibitors</td>
<td>Horie et al, 2004</td>
</tr>
<tr>
<td>LY29 and LY30</td>
<td>FIT Kinase inhibitor</td>
<td>Park et al, 2002</td>
</tr>
<tr>
<td>Shiga toxin (Entromorphagic E coli)</td>
<td>AKit-IKK interaction</td>
<td>Choi et al, 2004</td>
</tr>
<tr>
<td>Evodiamine (Eodiace Fructus component)</td>
<td>up-regulates Raf-1 kinase inhibitors</td>
<td>Gobert et al, 2007</td>
</tr>
<tr>
<td>Rituximab (anti-CD20 antibody)</td>
<td>MRRK3 inhibitor</td>
<td>Takada et al, 2005</td>
</tr>
<tr>
<td>Xylose suppressor of zas (ISPR2)</td>
<td></td>
<td>Jazireh et al, 2005</td>
</tr>
<tr>
<td>Cholecsytokinin c-peptide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(CCK-8)p38 kinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2L Vaccinia virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pefabloc (serine protease inhibitor) upstream of IKK</td>
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<td></td>
</tr>
<tr>
<td>Rocaglamides (Aglaia derivatives)</td>
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<td>Ysner</td>
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<td>Epoxyniquinol B</td>
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<td>Betaine</td>
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<td>TNAp</td>
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<td>Selected peptides</td>
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<td>Biochalin</td>
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<tr>
<td>Defluriane</td>
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<td>Geranamycin</td>
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TABLE G-continued
TABLE G-continued

Examples of NFKB Inhibitors

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Examples of NFKB Inhibitors

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<td>Protocatuechina Aldehyde</td>
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<td>20(S)-Protopanaxatriol (ginsenoside metabolite)</td>
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<td>Rengyolone</td>
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<td>Rottleria</td>
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<td>saikosaponin-d</td>
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<td>Saline (low Newtonic)</td>
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<td>Sanguijinine (pseudochelerythrine, 13-methyl[1,3]-benzodioxolo[5,6-c]-1,3-dioxolo-4,5-phenanthridinium)</td>
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<th>Degradation</th>
<th>Species/Mutant/Description</th>
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<td>Xue et al, 2005</td>
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<td>Chang et al, 2004</td>
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<td>Kim et al, 2002;</td>
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<td>Trouglogene (Farfarae finals)</td>
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<td>Kim et al, 2008</td>
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<td>U0126 (MEK inhibitor)</td>
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<td>Takaya et al, 2003</td>
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<td>Umbilical acetonic acid</td>
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Yulda-Hanso-Tang: Inhibitors from IMGENEX: NF-kB Pathway Inhibitory Peptides

<table>
<thead>
<tr>
<th>Cat.No</th>
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<tr>
<td>IMG-2000</td>
<td>IKKg NEMO Binding Domain (NBD) Inhibitory Peptide Set Functions as an IKKa/IKKbeta decoy by blocking to IKKg NBD, thereby preventing formation of the IKK complex.</td>
<td>H, M, R</td>
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Examples of NFKB Inhibitors

Any one or more of the following NFKB inhibitors

<table>
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<tr>
<th>Inhibitor</th>
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<tr>
<td>IKKg NEMO Binding Domain (NBD) Inhibitory Peptide</td>
<td>Set Functions as a decoy by binding to the IKKg NBD, thereby preventing formation of the IKK complex.</td>
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<tr>
<td>MyD88 Homodimerization Inhibitory Peptide</td>
<td>Set Functions as a decoy by binding to the MyD88 TIR domain.</td>
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<td>NF-kB p50 (NLS) Inhibitory Peptide</td>
<td>Set Functions as a decoy by blocking the intracellular recognition mechanism of p50 NLS.</td>
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<td>NF-kB p50 (NLS) Inhibitory Peptide</td>
<td>Set Functions as a decoy by blocking the intracellular recognition mechanism of p50 NLS.</td>
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<tr>
<td>NF-kB p65 (Ser276) Inhibitory Peptide</td>
<td>Set Functions as a p65 decoy through phosphorylation of the Ser276 site on the peptide.</td>
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<td>NF-kB p65 (Ser276) Inhibitory Peptide</td>
<td>Set Functions as a p65 decoy through phosphorylation of the Ser276 site on the peptide.</td>
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<tr>
<td>NF-kB p65 (Ser529/536) Inhibitory Peptide</td>
<td>Set Functions as a p65 decoy through phosphorylation of the Ser529/536 sites on the peptide.</td>
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<td>NF-kB p65 (Ser529/536) Inhibitory Peptide</td>
<td>Set Functions as a p65 decoy through phosphorylation of the Ser529/536 sites on the peptide.</td>
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<tr>
<td>TIRAP Inhibitory Peptide</td>
<td>Set Functions as a decoy by binding to TIR interacting domains on specific TLR receptors.</td>
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<tr>
<td>TLR4 Peptide Inhibitor Set: VIPER</td>
<td>Set Functions as a decoy by binding to TIR interacting domains on specific TLR receptors.</td>
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<tr>
<td>TRAF6 Inhibitory Peptide</td>
<td>Set Functions as a decoy by binding to the T6DP motif of RANK, thereby preventing binding of RANK to TRAF6.</td>
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<tr>
<td>TRAF6 Inhibitory Peptide</td>
<td>Set Functions as a decoy by binding to the T6DP motif of RANK, thereby preventing binding of RANK to TRAF6.</td>
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</table>

NF kappa B Inhibitors

- 9-Methylstreptimidone
- Z-VRPR-FMK
- 2-(1,8-naphthyridin-2-yl)-Phenol
- 5-Aminosalicylic acid
- BAY 11-7082
- BAY 11-7085
- Caffeic acid phenethyl ester
- Diethylmaleate
- Ethyl 3,4-Dihydroxyxycinnamate
- Helenalin
- NFICB Activation Inhibitor II, JSH-23
- NFICB Activation Inhibitor III
- PPM-18
- Pyrrolidinedithiocarbarnic acid ammonium salt
- (R)-MG-132
- Rocaglamide
- Sodium Salicylate
The presently-disclosed subject matter further includes compositions useful for inhibiting NFκB. Such compositions include an inhibitor. As noted above, such inhibitors can be, for example, a molecule, a polypeptide, a small (chemical) molecule, etc. In some embodiments, a composition can include an isolated RNA molecule.

The presently-disclosed subject matter further includes methods of screening candidate inhibitors to identify NFκB inhibitors. In some embodiments, cell or cell line-based methods are used.

Imaging Caspase in an Eye of a Subject

In some embodiments, a diagnostic composition is provided for imaging activated Caspase in an eye of a subject, comprising a fluorescent molecule conjugated to a substrate of Caspase-1 or a molecule that fluoresces following cleavage by Caspase-1. In some embodiments, a method is provided for imaging activated Caspase-1 in an eye of a subject, including administering (e.g., intracocularly or intraocularly) to RPE cells of the subject the diagnostic composition, and optically monitoring the spatial clustering of fluorescence.

The details of one or more embodiments of the presently-disclosed subject matter are set forth in this application. 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 particularly 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.

Some of the polynucleotide and polypeptide sequences disclosed herein are cross-referenced to GENBANK®/GENPEPT® accession numbers. The sequences cross-referenced in the GENBANK®/GENPEPT® database are expressly incorporated by reference as are equivalent and related sequences present in GENBANK®/GENPEPT® or other public databases. Also expressly incorporated herein by reference are all annotations present in the GENBANK®/GENPEPT® database associated with the sequences disclosed herein. Unless otherwise indicated or apparent, the references to the GENBANK®/GENPEPT® database are references to the most recent version of the database as of the filing date of this Application.

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 the 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, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about”. Accordingly, unless indicated to the contrary, the numerical parameters set forth in this specification and claims are approximations that can vary depending upon the desired properties sought to be obtained by the presently-disclosed subject matter.

As used herein, the term “about,” when referring to a value or to an amount of mass, weight, time, volume, concentration or percentage is meant to encompass variations of in some embodiments ±20%, in some embodiments ±10%, in some embodiments ±5%, in some embodiments ±1%, in some embodiments ±0.5%, and in some embodiments ±0.1% from the specified amount, as such variations are appropriate to perform the disclosed method.

As used herein, ranges can be expressed as from “about” one particular value, and/or to “about” another particular value. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as “about” that particular value in addition to the value itself. For example, if the value “10” is disclosed, then “about 10” is also disclosed. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

The presently-disclosed subject matter is further illustrated by the following specific but non-limiting examples. 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**

**Example 1**

Alu RNA accumulation due to DICER1 deficiency in the retinal pigmented epithelium (RPE) is implicated in geographic atrophy (GA), an advanced form of age-related macular degeneration that causes blindness in millions of individuals. The mechanism of Alu RNA-induced cytotoxicity is unknown. Here it is shown that DICER1 deficit or Alu RNA exposure activates the NLRP3 inflammasome and triggers TLR-independent MyD88 signaling via IL-18 in the RPE. Genetic or pharmacological inhibition of inflammasome components (NLRP3, Pyocard, Caspase-1), MyD88, or IL-18 prevents RPE degeneration induced by DICER1 loss or Alu RNA exposure. These findings, coupled with the observation that human GA RPE contains elevated amounts of NLRP3, PYCARD and IL-18, and evidence of increased Caspase-1 and MyD88 activation, provide a rationale for targeting this pathway in GA. The findings also reveal a novel function of the inflammasome outside the immune system and a surprising immunomodulatory action of mobile elements.

Age-related macular degeneration (AMD) affects the vision of millions of individuals (Smith et al., 2001). AMD is characterized by degeneration of the retinal pigmented epithelium (RPE), which is situated between the retinal photoreceptors and the choroidal capillaries (Ambati et al., 2003). RPE dysfunction disrupts both photoreceptors and choroidal vasculature (Blaauwgeers et al., 1999; Lopez et al., 1996; McLeod et al., 2000; Vogt et al., 2011). These tissue disruptions lead to atrophic or neovascular disease phenotypes. Although there are therapies for neovascular AMD, there is no effective treatment for the more common atrophic form, GA, the advanced stage of atrophic AMD, is characterized by degeneration of the RPE, and is the leading cause of treatable vision loss.
Recently it was shown that a dramatic and specific reduction of the RNase DICER1 leads to accumulation of Alu RNA transcripts in the RPE of human eyes with GA (Kaneko et al., 2011). These repetitive element transcripts, which are non-coding RNAs expressed by the highly abundant Alu retrotransposon (Batzer and Deininger, 2002), induce human RPE cell death and RPE degeneration in mice. DICER1 deficit in GA RPE was not a generic cell death response because DICER1 expression was not dysregulated in other retinal diseases. Likewise, Alu RNA accumulation did not represent generalized retrotransposon activation due to a stress response in dying cells because other retrotransposons were not elevated in GA RPE.

DICER1 is central to mature microRNA biogenesis (Bernstein et al., 2001). Yet following DICER1 deficit, the accumulation of Alu RNA and not the lack of mature microRNAs was the critical determinant of RPE cell viability (Kaneko et al., 2011). Moreover, 7SL RNA, transfer RNA, and primary microRNAs do not induce RPE degeneration (Kaneko et al., 2011), ruling out a nonspecific toxicity of excessive, highly structured RNA. Still, the precise mechanisms of Alu RNA cytotoxicity are unknown. Although the retina is exceptional for its immune privilege (Striekl, 2003), insults mediated by innate immune sensors can result in profound inflammation. The three major classes of innate immune receptors include the TLRs, RIG-I-like helicases, and NLR proteins (Akira et al., 2006). Numerous innate immune receptors are expressed in the RPE (Kumar et al., 2004), and several exogenous substances can induce retinal inflammation (Allensworth et al., 2011; Kleinman et al., 2012). However, it is not known whether this surveillance machinery recognizes or responds to host endogenous RNAs. The concept was explored that innate immune machinery, whose canonical function is the detection of pathogen associated molecular patterns and other moieties from foreign organisms, might also recognize Alu RNA.

Indeed, it was shown that Alu transcripts can hijack innate immunity machinery to induce RPE cell death. Surprisingly, the data show that DICER1 deficit or Alu RNA activates the NLRP3 inflammasome in a MyD88-dependent, but TLR-independent manner. NLRP3 inflammasome activation in vivo has been largely restricted to immune cells, although the data open the possibility that NLRP3 activity may be more widespread, as reflected by examples in cell culture studies of keratinocytes (Feldmeyer et al., 2007; Keller et al., 2008). The data also broaden the scope of DICER1 function beyond microRNA biogenesis, and identify it as a guardian against aberrant accumulation of toxic retrotransposon elements that comprise roughly 50% of the human genome (Lander et al., 2001). In sum, the findings present a novel self-recognition immune response, whereby endogenous non-coding RNA-induced NLRP3 inflammasome activation results from DICER1 deficiency in a non-immune cell.

Results

Alu RNA does not Activate a Variety of TLRs or RNA Sensors

Alu RNA has single-stranded (ss) RNA and double-stranded (ds) RNA motifs (Sinnett et al., 1991). Thus it was tested whether Alu RNA induced RPE degeneration in mice deficient in toll-like receptor-3 (TLR3), a dsRNA sensor (Alexopoulou et al., 2001), or TLR7, a ssRNA sensor (Diebold et al., 2004; Heil et al., 2004). Subretinal delivery of a plasmid coding for Alu RNA (pAlu) induced RPE degeneration in Tlr3-/- and Tlr7-/- mice just as in wild-type (WT) mice (FIGS. 1A-C). It was previously shown that ±21-nucleotide fully complementary siRNAs activate TLR3 on RPE cells (Kleinstein et al., 2011). Lack of TLR3 activation by Alu RNA is likely due to its complex structure containing multiple hairpins and bulges that might preclude TLR3 binding. Neither 7SL RNA, the evolutionary precursor of Alu RNA, nor p7SL induced RPE degeneration in WT mice (FIGS. 8A and 8B), suggesting that Alu RNA cytotoxicity might be due to as yet unclear structural features. pAlu induced RPE degeneration in Unc93b1 mice (FIG. 1D), which lack TLR3, TLR7, and TLR9 signaling (Tabeta et al., 2006), indicating that these nucleic acid sensors are not activated by Alu RNA redundantly. pAlu induced RPE degeneration in Tlr4-/- mice (FIG. 1E), and the TLR4 antagonist Rhodobacter sphaeroides LPS (Qureshi et al., 1991) did not inhibit pAlu-induced RPE degeneration in WT mice (FIG. 8C). Thus the observed RPE cell death is not due to lipopolysaccharide contamination. Further, two different in vitro transcribed Alu RNAs (Kaneko et al., 2011) did not activate multiple TLRs (FIG. 1F).

Next it was tested whether other dsRNA sensors such as MDA5 (Kato et al., 2006) or PKR (encoded by Prkr, (Yang et al., 1995)) might mediate Alu RNA toxicity. However, pAlu induced RPE degeneration in Mda5-/- and Prkr-/- mice (FIGS. 8D and 8E). It was tested whether the 5’-triphosphate on in vitro transcribed Alu RNA, which could activate RIG-I or IFIT-1 that sense this moiety (Hornung et al., 2006; Pichlmair et al., 2011), was responsible for RPE degeneration. Dephosphorylated Alu RNA induced RPE degeneration in WT mice just as well as Alu RNA not subjected to dephosphorylation (FIG. 8F), indicating that this chemical group is not responsible for the observed cell death. Indeed a 5’-triphosphate ssRNA that activates RIG-I does not induce RPE degeneration in mice (Kleinman et al., 2011). Further, pAlu induced RPE degeneration in mice deficient in MAVS (FIG. 8G), through which RIG-I and MDA-5 signal (Kumar et al., 2006; Sun et al., 2006). Collectively these data pointed to a novel mechanism of Alu RNA-induced RPE degeneration not mediated by a wide range of canonical RNA sensors.

Alu RNA Cytotoxicity is Mediated Via MyD88 and IL-18

The involvement of TRIF (encoded by TICAM1), an adaptor for TLR3 and TLR4 (Hoebe et al., 2003; Yamamoto et al., 2003), and MyD88, an adaptor for all TLRs except TLR3 (Akira et al., 2006; Alexopoulou et al., 2001; Suzuki et al., 2003) were then tested. Alu RNA induced RPE degeneration in Ticam1-/- mice (FIG. 9A), consistent with findings in Trif-/- and Trif4-/- mice. Unexpectedly, neither Alu RNA nor two different pAlu plasmids induced RPE degeneration in Myd88-/- mice (FIGS. 2A, 9B, and 9C). Intravenous delivery of a peptide inhibitor of MyD88 homodimerization (Loiarro et al., 2005) prevented RPE degeneration induced by Alu RNA in WT mice, whereas a control peptide did not do so (FIG. 2B). A MyD88-targeting short interfering RNA (siRNA), which was shorter than 21 nucleotides in length to prevent TLR3 activation and conjugated to cholesterol to enable cell permeation (Kleinman et al., 2008), but not a control siRNA, inhibited RPE degeneration induced by pAlu in WT mice (FIGS. 2F and 9D), corroborating the siRNA studies that partial knockdown of MyD88 is therapeutically sufficient.

MyD88-mediated signal transduction induced by interleukins leads to recruitment and phosphorylation of IRAK1 and IRAK4 (Cao et al., 1996; Kanakaraj et al., 1999; Suzuki et al., 2003; Suzuki et al., 2002). Alu RNA increased IRAK1/4 phosphorylation in human RPE cells (FIG. 2G), supporting the concept that Alu RNA triggers MyD88 sig-
naling. The MyD88 inhibitory peptide reduced Alu RNA-induced IRAK1/4 phosphorylation in human RPE cells (FIG. 9E), confirming its mode of action.

Next it was assessed whether MyD88 activation mediates Alu RNA-induced cell death in human and mouse RPE cell culture systems. Consonant with the in vivo data, pAlu reduced cell viability in WT but not MyD88−/− mouse RPE cells (FIG. 21i). The MyD88-inhibitory peptide, but not a control peptide, inhibited cell death in human RPE cells transfected with pAlu (FIG. 21). Together, these data indicate that MyD88 is a critical mediator of Alu RNA-induced RPE degeneration.

MyD88 is generally considered an adaptor of immune cells (O’Neill and Bowie, 2007). However, Alu RNA induced cell death via MyD88 in RPE monoculture. Thus, it was tested whether Alu RNA-induced RPE degeneration in mice was also dependent solely on MyD88 activation in RPE cells. Conditional ablation of MyD88 in the RPE by subretinal injection of AAV1-BEST1-Cre in MyD88−/− mice protected against Alu RNA-induced RPE degeneration (FIG. 2J and 9F). Consistent with this finding, Alu RNA induced RPE degeneration in WT mice receiving MyD88−/− bone marrow but did not do so in MyD88−/− mice receiving WT bone marrow (FIG. 9G). Collectively, these results indicate that MyD88 expression in the RPE, and not in circulating immune cells, is critical for Alu RNA-induced RPE degeneration. These findings comport with histopathological studies of human GA tissue that show no infiltration of immune cells in the area of pathology (personal communication, C. A. Curcio, H. E. Grossniklaus, G. S. Hageman, L. V. Johnson).

Although MyD88 is critical in TLR signaling (O’Neill and Bowie, 2007), MyD88 activation by Alu RNA was independent of TLR activation. Thus, other mechanisms of MyD88 involvement were examined. MyD88 can regulate IFN-γ signaling by interacting with IFN-γ receptor 1 (encoded by Ilng1) (Sun and Ding, 2006). However, pAlu induced RPE degeneration in both Ilng1−/− and Ilng1−/− mice (FIGS. 9H and 9I). MyD88 is also essential in interleukin-1 signaling (Muzio et al., 1997). Thus, it was tested whether IL-1β and the related cytokine IL-18, both of which activate MyD88 (Adachi et al., 1998), mediated Alu RNA cytotoxicity. Interestingly, whereas Alu RNA overexpression in human RPE cells increased IL-18 secretion, IL-1β secretion was barely detectable (FIG. 2K).

Recombinant IL-18 induced RPE degeneration in WT but not MyD88−/− mice (FIG. 2L). IL-18 neutralization protected against pAlu-induced RPE degeneration in WT mice, but IL-1β did not (FIGS. 2M and 2N). Also, pAlu induced RPE degeneration in Il1β−/− mice but not Il1β−/− mice (FIGS. 9J and 9K). These data indicate that IL-18 is an effector of Alu RNA-induced cytotoxicity.

Alu RNA Activates the NLRP3 Inflammasome

It was explored whether Caspase-1 (encoded by Casp1), a protease that induces maturation of interleukins into biologically active forms (Gayther et al., 1997; Gu et al., 1997; Thornberry et al., 1992), was involved in Alu RNA-induced RPE degeneration. Alu RNA treatment of human RPE cells led to Caspase-1 activation as measured by western blotting and by a fluorescent reporter of substrate cleavage (FIGS. 3A and 10A). Indeed, Alu RNA induced Caspase-1 activation in other cell types such as h1el.a and THP-1 monocytic cells (FIG. 10B), suggesting that Alu RNA cytotoxicity has potentially broad implications in many systems. Intravenous delivery of the Caspase-1-inhibitory peptide Z-WEHD-FMK, but not a control peptide Z-FA-FMK, blocked IL-18 maturation and pAlu-induced RPE degeneration in WT mice (FIGS. 3B and 3C). The Caspase-1-inhibitory peptide blocked Alu RNA-induced substrate cleavage in human RPE cells (FIG. 10C), confirming its mode of action. Similarly, Casp1−/− mice treated with Alu RNA or pAlu did not exhibit RPE degeneration (FIGS. 3D and 10D). Also, pAlu did not induce cell death in Casp1−/− mouse RPE cells (FIG. 3E).

Caspase-1 can be activated within a multiprotein innate immune complex termed the inflammasome (Tschopp et al., 2003). The best-characterized inflammasome pathway is one that is activated by binding of NLRP3 to the caspase-1 adaptor ASC (encoded by PYCARD). One hallmark of inflammasome assembly is spatial clustering of PYCARD (Fernandes-Alnemri et al., 2007). In human RPE cells transfected with fluorescent tagged PYCARD (GFP-PYCARD), Alu RNA induced the appearance of a brightly fluorescent cytoplasmic cluster similar to treatment with LPS and ATP, which activates the NLRP3 inflammasome (FIGS. 3F and 10E) (Mariathasan et al., 2006).

Next the functional relevance of NLRP3 and PYCARD to Alu RNA cytotoxicity was tested. Neither pAlu nor Alu RNA induced RPE degeneration in either Nlrp3−/− or PyCARD−/− mice (FIGS. 3G, 3H, 10F and 10G), demonstrating the critical importance of the inflammasome in Alu RNA cytotoxicity. Also, pAlu did not induce cell death in Nlrp3−/− or PyCARD−/− mouse RPE cells (FIG. 3I). Moreover, knock-down of NLRP3 or PYCARD by siRNAs rescued pAlu-induced human RPE cell death (FIGS. 3J and 10H). These findings provide direct evidence that NLRP3 activation in response to Alu RNA occurs in RPE cells and does not require the presence of other immune cells.

It was determined that IL-18 and MyD88 activation indeed were downstream of Caspase-1 activation by showing (1) that whereas MyD88 inhibition reduced Alu RNA-induced IRAK1/4 phosphorylation in human RPE cells (FIG. 9E), it did not reduce Alu RNA-induced Caspase-1 cleavage or fluorescent substrate cleavage (FIGS. 10I and 10J); (2) that IL-18 neutralization did not inhibit Alu RNA-induced Caspase-1 cleavage (FIG. 10K); and (3) that Caspase-1 inhibition reduced Alu RNA-induced phosphorylation of IRAK1/4 (FIG. 10L).

Alu RNA Induces Mitochondrial ROS and NLRP3 Priming

NLRP3 inflammasome function requires two signals, the first of which is termed priming, pAlu-induced inflammasome priming as it upregulated both NLRP3 and IL18 mRNAs. This priming occurred equivalently in both WT and MyD88−/− mouse RPE cells (FIG. 4A), further corroborating that MyD88 functions downstream of NLRP3 in this system. Akin to other inflammasome agonists that do not directly interact with NLRP3 (Tschopp and Schroder, 2010), the biological difference between Alu RNA and NLRP3 was not observed (FIG. 11A). To determine how Alu RNA primed the inflammasome, it was studied whether it induced reactive oxygen species (ROS) production, a signal for priming (Bauernfeind et al., 2011; Nakahira et al., 2011). pAlu induced ROS generation in human RPE cells (FIG. 4B), and the ROS inhibitor diphenylisodonium (DPI) blocked pAlu-induced NLRP3 and IL18 mRNA upregulation and Alu RNA-induced RPE degeneration in WT mice (FIGS. 4C and 4D). As DPI blocks mitochondrial ROS and phagosomal ROS (Li and Trush, 1998), it was tested which pathway was triggered because there is controversy surrounding the source of ROS contributing to NLRP3 responses (Latz, 2010).

MitoSOX Red was used, which labels ROS-producing mitochondria, in combination with MitoTracker Deep Red, which labels respiring mitochondria. To monitor phago-
sonal ROS generation. Fc OxyBURST Green was used, which measures activation of NADPH oxidase within the phagosome. A marked increase in ROS-generating mitochondria was observed in human RPE cells transfected with pAlu (FIG. 4E). In contrast, whereas phorbol myristate acetate (PMA) induced phagosomal ROS as expected (Savina et al., 2006), pAlu did not do so (FIG. 4F). These data are consistent with the findings that NLRP3 responses are impaired by mitochondrial ROS inhibitors (Nakahira et al., 2011; Zhou et al., 2011) but are preserved in cells carrying genetic mutations that impair NADPH-oxidase-dependent ROS production (Meissner et al., 2010; van Bruggen et al., 2010).

Consonant with these reports and the observation that the principal source of cellular ROS is mitochondria (Murphy, 2009), it was found that the mitochondria-targeted antioxidants Mito-TEMPO and MitoQ (Murphy and Smith, 2007; Nakahira et al., 2011) both blocked Alu RNA-induced RPE degeneration in WT mice, whereas dTTP, a structural analog of MitoQ that does not scavenge mitochondrial ROS, did not do so (FIG. 4G). In contrast, gp91ds-tat, a cell-permeable peptide that inhibits association of two essential NADPH oxidase subunits (gp91phox and p47phox) (Rey et al., 2001), did not do so (FIG. 4H). Corroborating these data, Alu RNA induced RPE degeneration in mice deficient in Cybb (which encodes gp91phox) just as in WT mice (FIG. 4I). Next the voltage-dependent anion channels (VDAC) was studied because VDAC1 and VDAC2, but not VDAC3, are important in mitochondrial ROS produced by NLRP3 activators in macrophages (Zhou et al., 2011). Consistent with these observations, siRNA knockdown of VDAC1 and VDAC2, but not VDAC3, impaired pAlu-induced mitochondrial ROS (FIGS. 4J and 11B) and NLRP3 and IL18 mRNA induction in human RPE cells (FIG. 4K). Collectively, these data implicate mitochondrial ROS in Alu RNA-induced NLRP3 inflammasome-mediated RPE degeneration.

Alu RNA does not induce RPE Degeneration Via Pyroptosis

Alu RNA activates Caspase-1, which can trigger pyroptosis, a form of cell death characterized by formation of membrane pores and osmotic lysis (Fink and Cookson, 2006). The cytoprotective agent glycine, which attenuates pyroptosis (Fink et al., 2008; Fink and Cookson, 2006; Verhoeef et al., 2005), inhibited human RPE cells death induced by LPS+ATP but not by Alu RNA (FIGS. 5A and 5B). Pyroptosis requires Caspase-1 but can proceed independent of IL-18 (Miao et al., 2010). Thus, the finding that IL-18 induced RPE degeneration in Casp1−/− mice (FIG. 5C), coupled with the lack of rescue by glycine, suggests that Alu RNA-induced RPE degeneration does not occur via pyroptosis.

Dicer1 Loss Induces Cell Death Via Inflammasome

It was previously demonstrated that the key role of Dicer1 in maintaining RPE cell health (Kaneko et al., 2011): Dicer1-cleaved Alu RNA did not induce RPE degeneration in vivo; Dicer1 overexpression protected against Alu RNA-induced RPE degeneration; and Dicer1 loss-induced RPE degeneration was blocked by antagonizing Alu RNA (Kaneko et al., 2011). Also, rescue of Dicer1 knockdown-induced RPE degeneration by Alu RNA inhibition was not accompanied by restoration of microRNA deficits (Kaneko et al., 2011). Therefore, it was tested whether Dicer1 also prevented NLRP3 inflammasome activation by Alu RNA. Alu RNA-induced Caspase-1 activation in human RPE cells was inhibited by Dicer1 overexpression (FIGS. 6A and 6B). Conversely, Caspase-1 cleavage induced by Dicer1 knockdown in human RPE cells was inhibited by simultaneous antisense knockdown of Alu RNA (FIGS. 12A and 12B).

Next the relevance of these pathways was tested in the context of Dicer1 loss in vivo. Caspase-1 cleavage was increased in the RPE of Best1Cre; Dicer1−/− mice (FIG. 6C), which lose Dicer1 expression in the RPE during development and exhibit RPE degeneration (Kaneko et al., 2011). Subretinal delivery of AAV1-Best1-Cre in Dicer1−/− mice induced Caspase-1 activation and IL-18 maturation in the RPE (FIG. 6D). This treatment also induced RPE degeneration, which was blocked by intravitreous delivery of the Caspase-1-inhibitory peptide but not the control peptide (FIG. 6E). AAV1-Best1-Cre-induced RPE degeneration in Dicer1−/− mice was also blocked by intravitreous delivery of the MyD88-inhibitory peptide but not a control peptide (FIG. 6F). In addition, MyD88 inhibition prevented cell death in human RPE cells treated with antisense oligonucleotides targeting Dicer1 (FIG. 6G). Dicer1 knockdown in human RPE cells increased IRAK1/4 phosphorylation, providing further evidence of MyD88 activation upon loss of Dicer1 (FIG. 6H). MyD88 inhibition also prevented cell death in Dicer1−/− mouse RPE cells treated with an adenoviral vector coding for Cre recombinase (FIG. 6I). MyD88 inhibition blocked RPE cell death without restoring the microRNA expression deficits induced by Dicer1 knockdown (FIG. 6J). These findings demonstrate that Dicer1 is an essential endogenous negative regulator of NLRP3 inflammasome activation, and that Dicer1 deficiency leads to Alu RNA-mediated, MyD88-dependent, microRNA-independent RPE degeneration.

Inflammasome and MyD88 Activation in Human GA

Next it was tested whether human eyes with GA, which exhibit loss of Dicer1 and accumulation of Alu RNA in their RPE (Kaneko et al., 2011), also display evidence of inflammasome activation. The abundance of NLRP3 mRNA in the RPE of human eyes with GA was markedly increased compared to control eyes (FIG. 7A). IL18 and IL1B mRNA abundance also was increased in GARPE; however, only the disparity in IL18 levels reached statistical significance (FIG. 7A). Immunolocalization studies showed that the expression of NLRP3, PYCARD, and Caspase-1 proteins was also increased in GA RPE (FIGS. 7B-D). Western blot analyses corroborated the increased abundance of NLRP3 and PYCARD in GA RPE, and revealed greatly increased levels of the enzymatically active cleaved Caspase-1 p20 subunit in GA RPE (FIG. 7E). There was also an increase in the abundance of phosphorylated IRAK1 and IRAK4 in GA RPE, indicative of increased MyD88 signal transduction (FIG. 7E). Collectively, these data provide evidence of NLRP3 inflammasome and MyD88 activation in situ in human GA, mirroring the functional data in human RPE cell culture and mice in vivo.

Discussion

The data establish a functional role for the subversion of innate immune sensing pathways by Alu RNA in the pathogenesis of GA. Collectively, the findings demonstrate that the NLRP3 inflammasome senses GA-associated Alu RNA danger signals, contributes to RPE degeneration, and potentially vision loss in AMD (FIG. 13). To date, the function of the NLRP3 inflammasome has been largely restricted to immune cells in vivo. The finding that it plays a critical function in RPE cell survival broadens the cellular scope of this inflammasome and raises the possibility that other non-immune cells could employ this platform.

The NLRP3 inflammasome was originally recognized as a sensor of external danger signals such as microbial toxins (Kane and Cookson, 2006; Mairathasan et al., 2006; Muruve...
et al., 2008). Subsequently, endogenous crystals, polypeptides, and lipids were reported to activate it in diseases such as gout, atherosclerosis, Alzheimer disease, and Type 2 diabetes (Halle et al., 2008; Masters et al., 2010; Murtveit et al., 2008; Wen et al., 2011). To the knowledge, Alu RNA is the first endogenous nucleic acid known to activate this immune platform. The findings expand the diversity of endogenous danger signals in chronic human diseases, and comport with the concept that this inflammasome is a sensor of metabolic danger (Schröder et al., 2010).

Dampening inflammasome activation can be essential to limiting the inflammatory response. Pathogens have evolved many strategies to inhibit inflammasome activation (Martinon et al., 2009). Likewise, host autophagy proteins (Nakahira et al., 2011), Type I interferon (Guarda et al., 2011), and T cell contact with macrophages can inhibit this process (Guarda et al., 2009). The finding that DICER1, through its cleavage of Alu RNA, prevents activation of NLPR3 adds to the repertoire of host inflammasome modulation capabilities and reveals a new facet of how dysregulation of homeostatic anti-inflammatory mechanisms can promote AMD (Ambati et al., 2003; Takada et al., 2009).

Added to its recently described anti-apoptotic and tumour-related functions, DICER1 emerges as a multifaceted protein. It remains to be determined how this functional versatility is channeled in various states. As DICER1 dysregulation is increasingly recognized in several human diseases, it is reasonable to imagine that Alu RNA might be an inflammasome activating danger signal in those conditions too. It is also interesting that, at least in adult mice and in a variety of mouse and human cells, the microRNA biogenesis function of DICER1 is not critical for cell survival, at least in a MyD88-deficient environment (data not shown).

The data that mitochondrial ROS production is involved in Alu RNA-induced RPE degeneration comport with observations of mitochondrial DNA damage (Lin et al., 2011), downregulation of proteins involved in mitochondrial energy production and trafficking (Nordgaard et al., 2008), and reduction in the number and size of mitochondria (Feher et al., 2006) in the RPE of human eyes with AMD. Jointly, these findings suggest a potential therapeutic benefit to interfering with mitochondrial ROS generation.

Current clinical programs targeting the inflammasome largely focus on IL-1β; presently there are no IL-18 inhibitors in registered clinical trials. However, the data indicate that IL-18 is more important than IL-1β in mediating RPE cell death in GA (similar to selective IL-18 involvement in a colitis model (Zaki et al., 2010)), pointing to the existence of regulatory mechanisms by which inflammasome activation bifurcates at the level of or just preceding the interleukin effectors. Although Caspase-1 inhibition could be an attractive local therapeutic strategy, caspase inhibitors can promote alternative cell death pathways, possibly limiting their utility (Vandenabeele et al., 2006).

MyD88 is best known for transducing TLR signaling initiated by pathogen associated molecular patterns (O'Neill and Bowie, 2007), although recently it has been implicated in human cancers (Ngo et al., 2011; Puente et al., 2011). The findings introduce an unexpected new function for MyD88 in effecting death signals from mobile element transcripts that can lead to retinal degeneration and blindness, and raise the possibility that MyD88 could be a central integrator of signals from other non-NLRP3 inflammasomes that also employ Caspase-1 (Schröder and Tschopp, 2010). Since non-canonical activation of MyD88 is a critical checkpoint in RPE degeneration in GA (FIG. 13), it represents an enticing therapeutic target. A potential concern is its important anti-microbial function in mice (O'Neill and Bowie, 2007). However, in contrast to MyD88−/− mice, adult humans with MyD88 deficiency are described to be generally healthy and resistant to a wide variety of microbial pathogens (von Bumann et al., 2008). MyD88-deficient humans have a narrow susceptibility range to pyogenic bacterial infections, and that too only in early childhood and not adult life (Picard et al., 2010). Moreover, as evident from the siRNA and MyD88−/− studies, partial inhibition of MyD88 is sufficient to protect against Alu RNA. Localized intraocular therapy, the current standard of care in most retinal diseases, would further limit the likelihood of adverse infectious outcomes. It is reasonable to foresee development of MyD88 inhibitors for prevention or treatment of GA.

**Experimental Procedures**

Subretinal injection and imaging. Subretinal injections (1 μl) were performed using a Pico-Injector (PL-I-100, Harvard Apparatus). Plasmids were transfected in vivo using 10% Neuroporter (Genlantis). Fundus imaging was performed on a TRC-50 IX camera (Topcon) linked to a digital imaging system (Sony). RPE flat mounts were immunolabeled using antibodies against zonula occludens-1 (Invitrogen).

mRNA Abundance.

Transcript abundance was quantified by real-time RT-PCR using an Applied Biosystems 7900 HT Fast Real-Time PCR system by the 2−ΔΔCt method.

Protein Abundance and Activity.

Protein abundance was assessed by Western blot analysis using antibodies against Caspase-1 (1:500; Invitrogen), pIκBα (1:500; Thermo Scientific), pIκBα (1:500, Abbomax), PYCARD (1:200, Santa Cruz Biotechnology), NLRP3 (1:500, Enzo Life Sciences) and Vinculin (1:1,000; Sigma-Aldrich). Caspase-1 activity was visualized using Caspaseox 1 E12D (Oncolmmunin) according to manufacturer's instructions.

**Mice**

All animal experiments were approved by institutional review committees and in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research. Wild-type C57BL/6J, Cybb−/−, Tlr3−/−, Tlr4−/− (C57BL/10ScNJ), Tfr−/− (Tiecam1−/−), Ilf6−/−, Ilfr−/−, Ihl1−/−, Ihl2−/−, MyD88−/−, and Dicer−/− mice were purchased from The Jackson Laboratory. Casp1−/−, Nbp3−/−, and Pycard−/− mice have been previously described (Kanneganti et al., 2006). Une93b1 mutant mice were generously provided by B. A. Beutler via K. Fitzgerald. MyD88−/− and Thr−/− mice were generously provided by S. Akira via T. Hawn and D. T. Golenbock. Mda5−/− mice were generously provided by M. Colonna. mice were generously provided by B. R. Williams and R. L. Silverman. Mavs−/− mice were generously provided by Z. Chen via K. Fitzgerald. For all procedures, anesthesia was achieved by intraperitoneal injection of 100 mg/kg ketamine hydrochloride (Ft. 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 TRC-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 age-matched patients without AMD were obtained from various eye banks. These diagnoses were confirmed by dilated ophthalmic examination prior to acquisition of the tissues or eyes upon
examination of the eye globes post mortem. The study followed the guidelines of the Declaration of Helsinki. Institutional review boards granted approval for all histological and histological analyses of specimens.

Immunolabeling. Human eyes fixed in 2-4% paraformaldehyde were prepared as eyecups, cryoprotected in 30% sucrose, embedded in optimal cutting temperature compound (Tissue-Tek OCT; Sakura Finetek), and cryosectioned into 10 μm sections. Depigmentation was achieved using 0.25% potassium permanganate and 0.1% oxalic acid. Immunohistochemical staining was performed with the rabbit antibody against NLRP3 (1:100, Sigma Aldrich) or rabbit antibody against Caspase-1 (prediluted, AbCam). Isootype IgG was substituted for the primary antibody to assess the specificity of the staining. Bound antibody was detected with biotin-conjugated secondary antibodies, followed by incubation with ABC reagent and visualized by Vector Blue (Vector Laboratories). Levamisole (Vector Laboratories) was used to inhibit nonspecific staining. Mouse RPE/choroid flat mounts were fixed with 4% paraformaldehyde and washed in PBS, counterstained with neutral red (Fisher Scientific), rinsed with deionized water, air dried, and then mounted in Vectamount (Vector Laboratories). Fluorescent labeling of human tissue was performed with the rabbit antibody against PYCARD (1:50, Clone N-15, Santa Cruz Biotechnology) Immunochemical labeling was visualized by fluorescently conjugated anti-rabbit secondary antibody (Invitrogen). Tissue autofluorescence was quenched by incubating the sections in 0.3% Sudan black (Fisher Scientific). Sections were mounted in Vectashield with DAPI (Vector Laboratories). Mouse RPE/choroid flat mounts were fixed with 4% paraformaldehyde or 100% methanol, stained with rabbit antibodies against human zonula occludens-1 (1:100, Invitrogen) and visualized with Alexa594 (Invitrogen). All images were obtained using the Leica SP-5 or Zeiss Axio Observer 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 two different Alu sequences (pAlu) or empty control vector (pNull) (Bennett et al., 2008; Kaneko et al., 2011; Shinkh et al., 1997) was achieved using 10% Neuroporter (Genlantis). AAV1-BEST1-Cre (Alexander and Hauswirth, 2008) or AAV1-BEST1-GFP were injected at 1.0×1011 pfu/mL and in vivo transcribed Alu RNA was injected at 0.3 mg/mL.

Drug Treatments. siRNAs formulated in siRNA buffer (20 mM Mgl2, 0.2 mM MgCl2 in HEPES buffer at pH 7.5; Dharmaco) or phosphate buffered saline (PBS; Sigma-Aldrich); the iL1R4 antagonist Ultra Pure Rhodobacter sphaeroides LPS (LPS-RS, InvivoGen), a peptide inhibitor of MyD88 homodimerization IMG-2005 (IMGENEX), control inhibitor (IMGENEX), recombinant IL-18 (Medical & Biological Laboratories), neutralizing rat antibodies against mouse IL-1β (IMGENEX), neutralizing rat antibodies against mouse IL-18 (Medical & Biological Laboratories), isotype control IgGs (R&D Systems or eBioscience as appropriate), Caspase-1 inhibitor Z-WEHD-FMK (R&D Systems), Caspase control inhibitor Z-FA-FMK (R&D Systems), DPI (Enzo Life Sciences), MitOQ and dTPP (both adsorbed to cyclodextrin and protected from cycloheximide), and gp91ds-tat and scrambled gp91 ds-tat (both Anaspec), were dissolved in phosphate buffered saline (PBS; Sigma-Aldrich) or dimethyl sulfoxide (DMSO; Sigma-Aldrich), and injected into the vitreous humor in a total volume of 1 μL with a 33-gauge Ex grote microinjection (Ito Corporation). To assess the effect of MyD88 blockade on pAlu-induced RPE degeneration, 1 μL of cholesterol (chol) conjugated MyD88 siRNA (17+2 nt; 2 μg/μL) was intravitreally injected 1 day after pAlu injection. As a control, luc siRNA-chol (17+2 nt) was used with identical dosages.

Bone Marrow Chimeras. Bone marrow transplantation was used to create Myd88−/− chimera mice wherein the genetic deficiency of Myd88 was confined to either circulating cells (Myd88−/−→ WT) or nonhematopoietic tissue (WT−→Myd88−/−). Briefly, bone marrows were collected from femur and tibia of congenic WT or Myd88−/− donor mice by flushing with RPMI1640. After two washing steps, cells were re suspended in RPMI1640. 1×10⁶ cells in 150 μL of RPMI1640 were injected into the tail vein of irradiated donor mice. Two chimera groups were generated: WT−→Myd88−/− (WT cells into Myd88−/− mice) and Myd88−/−→WT (Myd88−/− cells into WT mice). 2 months after bone marrow transfer, mice were injected subretinally with Alu RNA, vehicle, pAlu, or pNull, and monitored for RPE degeneration 7 days later.

Real-Time PCR. Total RNA was extracted from tissues or cells using Trizol reagent (Invitrogen) according to manufacturer’s recommendations, DNase treated and reverse transcribed (Quantitect, Qagen). 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 human IL-1β (forward 5'-TTAAAGCCGGCCTGACAGA-3' and reverse 5'-GCAGATCGACAGGAGTTCATTG-3'), human IL-18 (forward 5'-ATCACCTGACTCCGGGAGGA-3' and reverse 5'-AGACCGCAATGGTCGGAATC-3'), human NLRP3 (forward 5'-GCACCTGGTTGCAAAGCTGAA-3' and reverse 5'-TCTGACACATGTCGTTGTA-3'), human PYCARD (forward 5'-GGCCAGCCGCTCATTITATAGA-3' and reverse 5'-GTITTGTGACCTCCGGAATAG-3'), human VDAC1 (forward 5'-AAGTCTCAAATTCCGAGGATG-3' and reverse 5'-CTGGTCACAGGCAAGATTGCA-3'), human VDAC2 (forward 5'-CATGTCACATCAAGAAGCT-3' and reverse 5'-CTTGATGTCCTCCAAAGGAATT-3'), human VDAC3 (forward 5'-TTGACACGGCCACAATCCAAA-3' and reverse 5'-GGCCAAAGGGTTGTTTAC-3'), human 18S RNA (forward 5'-GGCAGTTGAAGATTAATGG-3' and reverse 5'-GGCTCAGTCTGGCAAAACCA-CAA-3'), mouse Myd88 (forward 5'-CACTCTGTTGTGGTCCATGG-3' and reverse 5'-AGGGTGGATGTGCAACTTTG-3'), mouse Nlrp3 (forward 5'-ATGCTGTTTCCGACATCTCT-3' and reverse 5'-AACATGGCGAGATGTGAC-3'), mouse Il18 (forward 5'-GACAGCTGTGTTGAGGAT-3' and reverse 5'-TGGATCCATTTCTCCAAAGAG-3'), and mouse 18S RNA (forward 5'-CCGCTTTGCTCGAGG-3' and reverse 5'-CCGCTACGCTGTTCGGAAG-3') were used.

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 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 also 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 Quantification.

Total RNA containing miRNAs was polyadenylated and reverse transcribed using universal primer using the All-in-One miRNA q-RT-PCR Detection Kit (GeneCopoeia) according to the manufacturer’s specifications using a universal reverse primer in combination with the following forward primers: mouse miR-184 (5’-TGGACGGA-GAAGTGAAGGGTGAGAGGGCGA-3’), and mouse miR-221/222 (5’-AGC-TACCCTGGCTACTGCGG-3’); mouse miR-320u (5’- AAAAGCTTTTGAGGGGGCGA-3’), and mouse miR-484 (5’-TCGGGCTCTCCCTCCCCAGAT-3’). miRNA levels were normalized to levels of U6 snRNA (5’-AAT-TCGTTAGAAGGAGGAGGGCGA-3’; and universal primer) using the All-In-One reverse primer in combination with the following method. Detection was achieved by SYBR green qPCR with the following conditions: 95°C for 10 min followed by 40 cycles of 95°C for 10 s, 60°C for 4 s, 72°C for 20 s. Amplicon specificity was assessed by melt curve analysis and unique bands by agarose gel electrophoresis.

Western Blotting.

Tissues or cells 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 NuPAGE Bis-Tris gels (Invitrogen) and transferred to Immun-Blot PVDF membranes (Bio-Rad). Cells were scraped in hot Laemmli buffer (62.5 mM Tris base, pH 6.8, 2% SDS, 5% 2-Mercaptoethanol, 10% Glycerol, 0.01% Bromophenol Blue). Samples were boiled and run on 4-20% NuPAGE Tris-Glycine gels (Invitrogen). The transferred membranes were blocked for 1 h at RT and incubated with antibodies against human Caspase-1 (1:500; Invitrogen), mouse Caspase 1 (1:500; MBL), NLRP3 (1:1000; Enzo Life Sciences), PYCARD (1:1000, RayBiotech), phospho-IRAK1 (S376) (1:500, Thermo Scientific), phospho-IRAK4 (T345) (1:500, AbboMax), DIcER1 (1:2000; Bethyl), MyD88 (1:1,000; Cell Signaling), and mouse IL-18 (1:2000; MBL) at 4°C overnight. Protein loading was assessed by immunoblotting using an anti-Vinculin antibody (1:1000, 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).

Cell Culture.

All cell cultures were maintained at 37°C and 5% CO2. Primary mouse RPE cells were isolated as previously described (Yang et al., 2009) and grown in Dulbecco Modified Eagle Medium (DMEM) supplemented with 20% FBS and standard antibiotics concentrations. Primary human RPE cells were isolated as previously described (Yang et al., 2008) and maintained in DMEM supplemented with 10% FBS and antibiotics. HeLa cells were cultured in RPMI 1640 medium supplemented with 10% FBS and antibiotics.

In vitro transcription of Alu RNAs. Two Alu RNAs were synthesized: a 281 nt Alu sequence originating from the cDNA clone TS 103 (Shaikh et al., 1997) and a 302 nt Alu sequence isolated from the RPE of a human eye with geographic atrophy. Linearized plasmids containing these Alu sequences with an adjacent 5’ T7 promoter were subjected to AmpliScribe™ T7-Flash™ Transcription Kit (Epicentre) according to the manufacturer’s instructions. DNase-treated RNA was purified using MEGAclear™ (Ambion), and integrity was monitored by gel electrophoresis. This yields single stranded RNAs that fold into a defined secondary structure identical to Pol III derived transcripts. Where indicated, transcription RNA was dephosphorylated using calf intestine alkaline phosphatase (Invitrogen) and repurified by Phenol:Chloroform:Isoamyl alcohol precipitation.

Transient Transfection.

Human or mouse RPE cells were transfected with pUC19, pAlu, pCdNA3.1/Dicer-FLAG, pCdNA3.1, Alu RNA, LNL.RP3 siRNA sense (5’-GUUGACGUACGU- GUCUCUGUdTdT-3’), PYCARD siRNA sense (5’-GAAGCUCU- UCAGUCUACAdTdT-3’), MyD88 siRNA sense (sense: 5’-AGGAGCAAGGAAGAGAAGUGAdTdT-3’), VDAC1 siRNA sense (5’-CGGAAUGCGACGCAAGUAdTdT-3’), VDAC2 siRNA sense (5’-CCCUGGAUGUGGGAGCCUdTdT-3’), VDAC3 siRNA sense (5’-CCGUCUAAGGAUUUGGCUAdTdT-3’), Dicer1 antisense oligonucleotide (AS) (5’-GUGCACGTTTGTGCTUUCUA-3’), control (for Dicer1) AS (5’-TTGGAAGCATACGTGTTACTGTA-3’), Alu AS (5’-CCCUGGTTCCACGGCATTCTCGCTCAGGCGAGTAG- CTGGGCTACATACGGCAGGCGGCGACACCTCCCGG- GCTAATTGGTTGATTTTTT-3’), control (for Alu) AS (5’-GCATTGCCGAGCTATGTCGTGCGCTTCACCACCTACTCCTGTA- TGCTGCCCAGGGC-3’) using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions.

Adenoviral Infection.

Cells were plated at density of 15x10^3/cm^2 and after 16 h, at approximately 50% confluence, were infected with AdCre or AdNull (Vector laboratories) with a multiplicity of infection of 1,000.

Cell Viability.

MTS assays were performed using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega) according to the manufacturer’s instructions. For examining the cytoprotective effect of glycine in Alu RNA induced cell death, human RPE cells were transfected with pNull/pAlu. At 6 h post-transfection the cells were incubated with complete media containing glycine (5 mM) or vehicle, and cell viability was assessed after 24 h. Similarly, human RPE cells primed with LPS (5 µg/ml for 6 h) were treated with ATP (25 µM) in the presence of glycine containing media (5 mM). 30 min post ATP cell viability was assessed as described above.

Caspase-1 activity. Caspase-1 activity was visualized by incubating cells with Casphaulx1E1D2 reagent (Oncolipin) according to the manufacturer’s instructions. Caspaulx1E1D2 signal was quantified reading the fluorescence (excitation 552 nm, emission 580 nm) using a Synergy H4 reader (Biotek). Quantification of fluorescence from images was performed by converting images into grayscale in Adobe Photoshop CSS, and measuring the integrated density of black and white images using Image software (NIH) (Bogdanovich et al., 2008).

ROS Production.

Cellular ROS production was assessed using the ROS-specific probe 2’7’-dichlorodihydrofluorescein diacetate (H2DCFDA, BioChemica, Fluka). Mitochondrial ROS production was assessed using MitoSOX™ Red (Invitrogen). Sub-confluent human RPE cells were transfected with pNull or pAlu. After 24 h cells were loaded for 10 min at 37°C with 10 µM H2DCFDA or MitoSOX™ Red (Invitrogen) mitochondrial superoxide indicator for live-cell imaging and washed twice with PBS. H2DCFDA, fluorescence was recorded in 96-well plate using a Synergy 4 reader (Biotek) using a FITC filter (excitation 485 nm, emission 538 nm). To visualize respiring mitochondria for colocalization with the mitochondrial ROS signal, after PBS wash
cells were incubated with MitoTracker Deep Red™ (Invitro- 
gen) for 30 min at 37°C, and then washed twice with PBS. 
The fluorescent signals were detected using Leica SP-5 or 
Zeiss Axio Observer Z1 microscopes. Phagosomal ROS 
production was assessed using the Fe-OXYBURST Green™ 
assay (Invitrogen). Sub-confluent human RPE cells were 
transfected with pNull or pAlu, or treated with PMA (0.5 
µg/ml; Sigma-Aldrich). The cells were incubated with 
Krebs-Ringer's PBS (KRP) at 37°C for 20 min before 
adding Fe-OXYBURST Green™ with a Synergy 4 reader (Biotek) 
using FITC filter (excitation 485 nm, emission 538 nm).

RNA-Binding Protein Immunoprecipitation (RIP): 
The physical interaction between NLRP3 and Alu RNA 
was examined using RNA ChIP-IT kit following the manu-
facturer's instructions (Active Motif). Briefly, human RPE 
cells were transfected with pAlu and pNLRP3-FLAG (pro-
vided by G. Núñez) and the protein-RNA complexes were 
immunoprecipitated with antibodies against NLRP3 (Enzo 
Life Sciences), FLAG (Sigma-Aldrich) or control IgG 
(Sigma-Aldrich). RNA isolated from these immunoprecipi-
tates was analyzed by real-time RT-PCR using Alu-specific 
primers.

ELISA. 
Secreted cytokine content in conditioned cell culture 
media was analyzed using the Human IL-1β and IL-18 
ELISA Kits (R&D) according to the manufacturer’s instruc-
tions.

TLR Screen. 
A custom TLR ligand screen was performed by Invivo-
Gen using HEK293 cells over-expressing individual TLR 
family members coupled with an AP-1/IFN-κB reporter 
system. Cells were stimulated with each of two Alu RNAs 
synthesized by in vitro transcription, or a TLR-specific 
positive control ligand.

Statistics. 
Results are expressed as mean±SEM, with p<0.05 con-
sidered 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.

Example 2

It was shown that both in vitro transcribed Alu RNA and 
a plasmid encoding Alu (pAlu) both induce RPE cell death 
by inducing IL-18 secretion, which triggers MyD88-dependent 
signaling that leads to Caspase-3 activation. Determine 
the intervening mechanistic steps in this cell death pathway 
were sought.

Caspase-8 is known to activate Caspase-3 (Stennicke et al. 
1998). Therefore, it was tested whether Caspase-8 inhi-
bition would inhibit RPE cell death or degeneration induced 
by Alu RNA or pAlu. It was found that the Caspase-8 
inhibitory peptide Z-IETD-FMK, but not the control peptide 
Z-FA-FMK, blocked RPE degeneration induced by pAlu in 
wild-type mice (FIG. 14). Z-IETD-FMK also inhibited 
human RPE cell death induced by Alu RNA (FIG. 15) or 
pAlu (FIG. 16). It was also found that subretinal injection of 
recombinant IL-18 induced activation of Caspase-8, as 
demonstrated by a fluorometric assay, in the RPE of wild-type 
mice (FIG. 17). These data indicate that Alu RNA- 
or pAlu-induced IL-18 leads to Caspase-8 activation upstream 
of Caspase-3 activation.

MyD88 is known to bind Fas-associated death domain 
protein (FADD) and induce apoptosis via Caspase 8 (Alp-
arrants et al. 2000). Therefore, it was tested whether ablation 
of Fas (encoded by CD95) or Fasl. (encoded by Faslg) 
would inhibit RPE cell death or degeneration induced by Alu 
RNA, pAlu, or IL-18. It was found that neither pAlu (FIG. 
18) nor Alu RNA (FIG. 19) induced RPE degeneration in 
CD95−/− (Fas−/−) mice. In addition, recombinant IL-18 also 
did not induce RPE degeneration in CD95−/− (Fas−/−) mice 
(FIG. 20). Likewise, pAlu (FIG. 21), Alu RNA (FIG. 22), 
and IL-18 (FIG. 23) did not induce RPE degeneration in 
Fasl−/− (Fasl−/−) mice.

It has been shown that Alu RNA induces RPE degeneration 
via the NLRP3 inflammasome, because NF-κB activation 
is required for NLRP3 activation (Bauernfeind et al. 
2009; Qiao et al. 2012), it was tested whether Alu RNA 
required NF-κB to induce RPE degeneration. Indeed, it was 
found that Alu RNA did not induce RPE degeneration in 
Nfkb1−/− mice, confirming that NF-κB activation is a 
critical step in this cell death pathway.

Experimental Procedures

Mice. 
All animal experiments were approved by institutional 
review committees and in accordance with the Association 
for Research in Vision and Ophthalmology Statement for the 
Use of Animals in Ophthalmic and Visual Research. Wild-
type C57BL/6J, Fas−/− (a.k.a CD95 or Fas(p55) Fasl−/− (a.k.a. 
Fas(80)) and Nfkb1−/− mice were purchased from The Jackson 
Laboratory. For all procedures, anesthesia was achieved by 
intraperitoneal injection of 100 mg/kg ketamine hydrochlo-
ride (Fl. 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 TRC-50 IX camera (Topcon) linked to a digital 
imaging system (Sony).

Subretinal Injection. 
Subretinal injections (1 µl) in mice were performed using a 
Pico-Injector (PLI-100, Harvard Apparatus). In vivo trans-
faction of plasmids coding for two different Alu sequences 
(pAlu) or empty control vector (pNull) (Bennett et al., 2008; 
Kaneko et al., 2011; Shaikh et al., 1997) was achieved using 
10% Neuroporter (Genlanlts). In vitro transcribed Alu RNA 
was injected at 0.3 µg/mL.

Drug Treatments. 
Recombinant IL-18 (Medical & Biological Laboratories), 
Caspase-8 inhibitor Z-IETD-FMK (R&D Systems), Caspase 
control inhibitor Z-FA-FMK (R&D Systems), IRAK1/4 
inhibitor (Calbiochem), were dissolved in phosphate buff-
ered saline (PBS; Sigma-Aldrich) or dimethyl sulfoxide 
(DMSO; Sigma-Aldrich), and injected into the vitreous 
humor in a total volume of 1 µl with a 33-gauge L’xmine 
microsyringe (Ito Corporation).

Cell Culture. 
All cell cultures were maintained at 37°C in 5% CO2. 
Primary mouse RPE cells were isolated as previously 
-described (Yang et al., 2009) and grown in Dulbecco Modi-
fied Eagle Medium (DMEM) supplemented with 20% FBS 
and standard antibiotics concentrations. Primary human 
RPE cells were isolated as previously described (Yang et al., 
2008) and maintained in DMEM supplemented with 10% 
FBS and antibiotics. HeLa cells were maintained in DMEM 
supplemented with 20% FBS and standard antibiotics con-
centrations. THP-1 cells were cultured in RPMI 1640 
medium supplemented with 10% FBS and antibiotics.

In Vitro Transcription of Alu RNAs. 
We synthesized a 302 nt Alu sequence isolated from the 
RPE of a human eye with geographic atrophy. A linearized 
plasmid containing this Alu sequence with an adjacent 5’ T7
promoter were subjected to AmpliScribe™ T7-Flash™ Transcription Kit (Epicentre) according to the manufacturer’s instructions. DNase-treated RNA was purified using MEGAclear™ (Ambion), and integrity was monitored by gel electrophoresis. This yields single stranded RNAs that fold into a defined secondary structure identical to Pol III derived transcripts. Where indicated, transcribed RNA was dephosphorylated using calf intestine alkaline phosphatase (Invitrogen) and repurified by Phenol:Chloroform:Isoamyl alcohol precipitation.

Transient Transfection.

Human RPE cells were transfected with pUC19, pAlu, Alu RNA, VDAC1 siRNA sense (5'-CGGAAUAGCAGC-CAAG UdIdT-3'), VDAC2 siRNA sense (5'-CCCUG-GAGUUGGAGC UdIdT-3'), VDAC3 siRNA sense (5'-GCUUUAAUCAUGGCAAdTdT-3'), using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions.

Cell Viability.

MTS assays were performed using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega) according to the manufacturer’s instructions.

Caspase-8 Activity.

RPE 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. The caspase-3 activity was measured using Caspase-8 Fluorimetric Assay (R&D) in accordance to the manufacturer’s instructions.

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.

Methods for Caspase Imaging.

Alu RNA or recombinant IL-18 was injected into the subretinal space of wild-type mice on day 0. DyeLight 782-VAD-FMK3 (ThermoScientific), a probe that fluoresces in the presence of bioactive caspsases, was injected into the vitreous humor of wild-type mice on day 2 or day 3 after injection.

Flat Mount Imaging.

At 24 hours after injection of DyeLight 782-VAD-FMK3, the eyecup was dissected out of the mice, the neural retina was removed, and a flat mount of the RPE was prepared, and viewed under a fluorescent microscope.

In Vivo Bioimaging in the Living Eye.

At intervals from 0-24 hours after injection of DyeLight 782-VAD-FMK3, fundus photographs were taken with the Topcon 50IX camera using the ICG filter.

Throughout this document, various references are mentioned. All such references are incorporated herein by reference to the same extent as if each individual reference was specifically and individually indicated to be incorporated by reference, including the references set forth in the following list:

REFERENCES


gamma inducing factor mediated by interleukin-1beta converting enzyme. Science 275, 206-209.


89) Latz, E.
50 the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol 12, 222-230.


153) Stasinakova, J. et al. Influenza A mutant viruses with altered NS1 protein function provoke caspase-1 activation in primary human macrophages, resulting in fast apopto-
sis and release of high levels of interleukins 1beta and 18.


173) TEMPO and its hydroxylamine Free Radic Res 43, 4-12.


180) Verhoef, P. A., Kertesy, S. B., Lundberg, K., Kahlen­


182) tion, and cytolysis by the P2X7 receptor. J Immunol 175, 7623-7634.


205) U.S. Provisional Patent Application No. 61/586,427, filed Jan. 13, 2012. 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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What is claimed is:

1. A method of protecting a retinal pigment epithelium (RPE) cell, a retinal photoreceptor cell, or a choroidal cell, comprising:
   
   - inhibiting one or more of an inflammasome of the cell;
   - MyD88 of the cell; IL-18 of the cell; VDAC1 of the cell; VDAC2 of the cell; caspase-8 of the cell; and NfkB of the cell;
   - wherein the cell is protected against Alu-RNA-induced degeneration;
   
   wherein inhibiting the inflammasome consists essentially of administering an inflammasome inhibitor selected from the group consisting of an NLRP3 inhibitor, a PYCARD inhibitor, an inflammasome inhibitor comprising a sequence selected from the sequences of SEQ ID NOS: 7-16, an inhibitor of Caspase-1, a peptide inhibitor of Caspase-1, a Caspase-1 inhibitor comprising the sequence of SEQ ID NO: 17, and the inflammasome inhibitors selected from: ion channel inhibitors; IkB-α inhibitors; antibodies selected from: Anti-ASC; Anti-NALP1, antibodies based on protein sequences selected from: ASC: ALR QTQ PYL VTD LEQ S; NALP1: MEE SQS KEE SNT EG-cys; Anti-NALP1, anti-II-1β, anti-II-18, anti-caspase-1, and anti-caspase-11; direct inhibitors of Caspase-1 and/or NLRP3; potassium chloride; Cathepsin-B-inhibitors; Cytochalsin D; ROS inhibitors; ASC-1 inhibitors; NLRP3 inflammasome pan-caspase inhibitors; Microtubules; an isolated double-stranded RNA molecule that inhibits expression of NLRP3, and which can be conjugated to cholesterol or not, and at least one strand including the sequence: GUUJUGACUAUCUGUUCLdTdT (SEQ ID NO: 7), an isolated double-stranded RNA molecule that inhibits expression of PyCARD, at least one strand of which includes the sequence of: 5'-GAAGCUCUUCAGUUUCAdTdT-3' (SEQ ID NO: 12); and an isolated double-stranded RNA molecule that inhibits expression of PyCARD, at least one strand of which includes a sequence selected from: 5'-GAAGCUCUUCAGUUUCAdTdT-3' (SEQ ID NO: 12); 5'-GGCCUGUGAGAUGCU-GUAACGGAAA-3' (SEQ ID NO: 13); and 5'-UUCCGUAACAGACACAGACGC-3' (SEQ ID NO: 14).

2. The method of claim 1, wherein the inhibiting MyD88 comprises administering a MyD88 inhibitor.

3. The method of claim 2, wherein the inhibitor is selected from the group consisting of a MyD88 inhibitor comprising a polypeptide sequence selected from the sequences of SEQ ID NO: 1, 54, 55, 60, and 61; and a MyD88 inhibitor comprising a double-stranded RNA molecule, at least one strand of which includes a sequence selected from SEQ ID NOS: 3, 4, 5, 6, and 56.

4. The method of claim 2, wherein the MyD88 inhibitor is selected from the group consisting of a MyD88 homodimerization inhibitor; Pep-MyD; a dominant negative or splice variant of MyD88; a MyD88 splice variants that lack exon 2; and MyD88 inhibitors as set forth in Table C.

5. The method of claim 4, wherein the inhibitor is administered by intravitreous injection; subretinal injection; episcleral injection; sub-tenon’s injection; retrobulbar injection; peribulbar injection; topical eye drop application; release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

6. A method of protecting an RPE cell, a retinal photoreceptor cell, or a choroidal cell, comprising:

   - inhibiting one or more of an inflammasome of the cell;
   - MyD88 of the cell; IL-18 of the cell; VDAC1 of the cell; VDAC2 of the cell; caspase-8 of the cell; and NfkB of the cell;
   - wherein the cell is protected against Alu-RNA-induced degeneration;

   wherein inhibiting the inflammasome comprises administering an inflammasome inhibitor selected from the group consisting of an NLRP3 inhibitor, a PYCARD inhibitor, an inflammasome inhibitor comprising a sequence selected from the sequences of SEQ ID NOS: 7-16, an inhibitor of Caspase-1, a peptide inhibitor of Caspase-1, a Caspase-1 inhibitor comprising the sequence of SEQ ID NO: 17, and the inflammasome inhibitors selected from: ion channel inhibitors; IkB-α inhibitors; antibodies selected from: Anti-ASC; Anti-NALP1, antibodies based on protein sequences selected from: ASC: ALR QTQ PYL VTD LEQ S; NALP1: MEE SQS KEE SNT EG-cys; Anti-NALP1, anti-II-1β, anti-II-18, anti-caspase-1, and anti-caspase-11; direct inhibitors of Caspase-1 and/or NLRP3; potassium chloride; Cathepsin-B-inhibitors; Cytochalsin D; ROS inhibitors; ASC-1 inhibitors; NLRP3 inflammasome pan-caspase inhibitors; Microtubules; an isolated double-stranded RNA molecule that inhibits expression of NLRP3, and which can be conjugated to cholesterol or not, and at least one strand including the sequence: GUUJUGACUAUCUGUUCLdTdT (SEQ ID NO: 7), an isolated double-stranded RNA molecule that inhibits expression of PyCARD, at least one strand of which includes the sequence of: 5'-GAAGCUCUUCAGUUUCAdTdT-3' (SEQ ID NO: 12); and an isolated double-stranded RNA molecule that inhibits expression of PyCARD, at least one strand of which includes a sequence selected from: 5'-GAAGCUCUUCAGUUUCAdTdT-3' (SEQ ID NO: 12); 5'-GGCCUGUGAGAUGCU-GUAACGGAAA-3' (SEQ ID NO: 13); and 5'-UUCCGUAACAGACACAGACGC-3' (SEQ ID NO: 14).
The method of any one of claim 18, wherein the inhibiting Caspase-8 inhibitor is selected from the group consisting of Z-IETD-FMK, Ac-Ala-Ala-Val-Ala-Leu-Leu-Pro-Ala-Val-Leu-Leu-Ala-Leu-Leu-Ala-Pro-Ile-Glu-Thr-Asp-CHO, Z-Ile-Glu (OMe)-Thr-Asp(OMe)-CH₂₂ and Cellular fas-associated death domain-like interleukin-1β-converting enzyme-inhibitory protein (L).

20. The method of any one of claim 18, wherein the inhibiting Caspase-8 inhibitor is selected from the group consisting of Z-IETD-FMK, Ac-Ala-Ala-Val-Ala-Leu-Leu-Pro-Ala-Val-Leu-Leu-Ala-Leu-Leu-Ala-Pro-Ile-Glu-Thr-Asp-CHO, Z-Ile-Glu (OMe)-Thr-Asp(OMe)-CH₂₂ and Cellular fas-associated death domain-like interleukin-1β-converting enzyme-inhibitory protein (L).

21. The method of claim 1, wherein the inhibiting NFkB inhibitor comprises administering a NFkB inhibitor.

22. The method of claim 21, wherein the inhibiting NFkB inhibitor is selected from intravitreous injection; subretinal injection; episcleral injection; sub-Tenon's injection; retrobulbar injection; peribulbar injection; topical eye drop application; release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

23. The method of claim 1, wherein the inhibiting VDAC1 inhibitor comprises administering a VDAC1 inhibitor and inhibiting VDAC2 comprises administering a VDAC2 inhibitor.

24. The method of claim 23, wherein the inhibiting NFkB inhibitor comprises administering a NFkB inhibitor.

25. The method of claim 24, wherein the inhibiting NFkB inhibitor is selected from intravitreous injection; subretinal injection; episcleral injection; sub-Tenon’s injection; retrobulbar injection; peribulbar injection; topical eye drop application; release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

26. The method of any one of claim 18, wherein the inhibiting IL-18 comprises administering an IL-18 inhibitor.

27. The method of claim 26, wherein the inhibiting IL-18 inhibitor is selected from the group consisting of a neutralizing antibody against IL-18; an antibody that blocks IL-18 binding to the IL-18 receptor, IL-18 neutralizing antibodies; IL-18-binding protein; and IL18BP.

28. The method of claim 13, wherein the inhibiting Caspase-8 inhibitor is selected from intravitreous injection; subretinal injection; episcleral injection; sub-Tenon’s injection; retrobulbar injection; peribulbar injection; topical eye drop application; release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

29. The method of any one of claim 18, wherein the inhibiting IL-18 inhibitor comprises administering an IL-18 inhibitor.

30. The method of claim 29, wherein the inhibiting IL-18 inhibitor is selected from the group consisting of a neutralizing antibody against IL-18; an antibody that blocks IL-18 binding to the IL-18 receptor, IL-18 neutralizing antibodies; IL-18-binding protein; and IL18BP.

31. The method of claim 1, wherein the inhibiting VDAC1 inhibitor comprises administering a VDAC1 inhibitor and inhibiting VDAC2 comprises administering a VDAC2 inhibitor.

32. The method of any one of claim 18, wherein the inhibiting NFkB inhibitor comprises administering a NFkB inhibitor.

33. The method of claim 32, wherein the inhibiting NFkB inhibitor is selected from intravitreous injection; subretinal injection; episcleral injection; sub-Tenon’s injection; retrobulbar injection; peribulbar injection; topical eye drop application; release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

34. The method of any one of claim 18, wherein the inhibiting IL-18 inhibitor comprises administering an IL-18 inhibitor.

35. The method of claim 34, wherein the inhibiting IL-18 inhibitor is selected from the group consisting of a neutralizing antibody against IL-18; an antibody that blocks IL-18 binding to the IL-18 receptor, IL-18 neutralizing antibodies; IL-18-binding protein; and IL18BP.

36. The method of claim 1, wherein the inhibiting Caspase-8 inhibitor comprises administering a Caspase-8 inhibitor.

37. The method of any one of claim 18, wherein the inhibiting NFkB inhibitor comprises administering a NFkB inhibitor.

38. The method of claim 37, wherein the inhibiting NFkB inhibitor is selected from intravitreous injection; subretinal injection; episcleral injection; sub-Tenon’s injection; retrobulbar injection; peribulbar injection; topical eye drop application; release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

39. The method of any one of claim 18, wherein the inhibiting IL-18 inhibitor comprises administering an IL-18 inhibitor.

40. The method of claim 39, wherein the inhibiting IL-18 inhibitor is selected from the group consisting of a neutralizing antibody against IL-18; an antibody that blocks IL-18 binding to the IL-18 receptor, IL-18 neutralizing antibodies; IL-18-binding protein; and IL18BP.

41. The method of claim 1, wherein the inhibiting VDAC1 inhibitor comprises administering a VDAC1 inhibitor and inhibiting VDAC2 comprises administering a VDAC2 inhibitor.

42. The method of any one of claim 18, wherein the inhibiting NFkB inhibitor comprises administering a NFkB inhibitor.

43. The method of claim 42, wherein the inhibiting NFkB inhibitor is selected from intravitreous injection; subretinal injection; episcleral injection; sub-Tenon’s injection; retrobulbar injection; peribulbar injection; topical eye drop application; release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

44. The method of any one of claim 18, wherein the inhibiting IL-18 inhibitor comprises administering an IL-18 inhibitor.

45. The method of claim 44, wherein the inhibiting IL-18 inhibitor is selected from the group consisting of a neutralizing antibody against IL-18; an antibody that blocks IL-18 binding to the IL-18 receptor, IL-18 neutralizing antibodies; IL-18-binding protein; and IL18BP.

46. The method of claim 1, wherein the inhibiting Caspase-8 inhibitor comprises administering a Caspase-8 inhibitor.

47. The method of any one of claim 18, wherein the inhibiting NFkB inhibitor comprises administering a NFkB inhibitor.

48. The method of claim 47, wherein the inhibiting NFkB inhibitor is selected from intravitreous injection; subretinal injection; episcleral injection; sub-Tenon’s injection; retrobulbar injection; peribulbar injection; topical eye drop application; release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.
release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

23. The method of claim 1, wherein the cell is in a subject.

24. The method of claim 23, wherein the subject has age-related macular degeneration.

25. An molecule selected from the group consisting of:
   an isolated double-stranded RNA molecule that inhibits expression of MyD88, wherein a first strand of the double-stranded RNA comprises a sequence selected from SEQ ID NO: 3, 4, 5, 6, and 56, and including about 11 to 27 nucleotides;
   an isolated double-stranded RNA molecule that inhibits expression of NLRP3 and/or PYCARD, wherein a first strand of the double-stranded RNA comprises a sequence selected from SEQ ID NO: 7-14, and including about 11 to 27 nucleotides;
   an isolated double-stranded RNA molecule that inhibits expression of Pyrin, comprising the sequence of SEQ ID NO: 15, and including about 11 to 27 nucleotides;
   an isolated double-stranded RNA molecule that inhibits expression of NALP3, comprising the sequence of SEQ ID NO: 16, and including about 11 to 27 nucleotides;
   an isolated double-stranded RNA molecule that inhibits expression of caspase-1, comprising the sequence of SEQ ID NO: 17, and including about 11 to 27 nucleotides;
   an isolated double-stranded RNA molecule that inhibits expression of VDAC1 and/or VDAC2, wherein a first strand of the double-stranded RNA comprises a sequence selected from SEQ ID NO: 47 and 48, and including about 11 to 27 nucleotides;

26. A polypeptide molecule that inhibits MyD88, comprising a sequence selected from SEQ ID NO: 60 and SEQ ID NO: 61.

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