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MicroRNAs: -the new frontier in personalized medicine for AMD?

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To learn more about the Kaarniranta AMD Lab

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This invited mini-review summarizes the most recent and promising results in the field and gives an overview of the future perspective of personalized miRNA AMD therapy.

Abstract

Since their discovery and the advent of epigenetic biology, microRNAs (miRNAs) have drawn enormous attention because of their ubiquitous involvement in cellular pathways, from life to death. From metabolism to communication, they are fundamental regulators linking all the body's biological functions. Using multiple techniques, researchers have already begun amassing information about miRNAs as biomarkers for different diseases, modulators of drug resistance, and as actual drugs to develop new personalized treatments for serious health conditions. Structurally, miRNAs are short non-coding RNAs involved in the control of protein and RNA production. They do this by altering gene expression levels and how the genetic information gets converted into proteins. The nature of this class of RNAs might make them particularly attractive drug targets for diseases with multifactorial origins, like age-related macular degeneration (AMD). It has been proposed that the reduction in the efficacy of the autophagic clearance in cells due to aging might be important in the pathogenesis of AMD. Autophagy is how a cell cleans up its waste products and recycles their materials. Several miRNAs have recently been detected to be dysregulated in AMD patients, and many of them regulate autophagy as well. The manipulation of miRNAs to strengthen autophagy might be a viable strategy to design new therapeutic approaches for AMD.

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Causes of age-related macular degeneration

The cellular pathology in AMD is strongly associated with oxidative stress, energy metabolism disturbances, protein aggregation, inflammation, and, in certain cases, neovascularization.[1-4] Particularly, oxidative stress-induced damage to the retinal pigment epithelium (RPE) is considered a key factor in AMD pathogenesis. There is increasing evidence that proteostasis is disturbed in the RPE, which, combined with oxidative stress, may lead to the accumulation of damaged cellular proteins, lipids, nucleic acids, and cellular organelles. This is evidenced by the accumulation of lysosomal lipofuscin and extracellular drusen, which are clinical hallmarks of AMD (**Figure 1**).[1]

Autophagy

Macroautophagy, or simply autophagy, is a cellular mechanism for clearing away damaged or unneeded components. It supports the degradation and recycling of

materials such as misfolded or too-long-lived proteins, lipids, protein aggregates, non-functional cellular organelles, and pathogens. In addition, autophagy is connected to the regulation of cell death. Autophagy is an important cellular mechanism which is involved in various cardiovascular and neurodegenerative diseases and cancers, as well as viral and bacterial infections. It is also vital in starvation situations, where cells need to literally eat themselves for sustenance.

The stages of autophagy (**Figure 2**) include engulfing the material to be decomposed by the phagophore membrane (induction/initiation and nucleation), the formation of the autophagosome (membrane elongation), its maturation, and transfer. The autophagosome fuses with the lysosome, which releases enzymes into the autophagosome, which degrade its contents.[5]

The weakening of autophagic clearance manifests as problems in cargo recognition, faults in autophagosome formation, disturbances in microtubule-assisted transport of autophagosomes, and failings of autophagosome-lysosome fusion and lysosome acidification.[6-7] This might be linked with the ageing of the cell and the regulation of cell death.[6] Some signs of autophagic malfunction include the downregulation of the expression of autophagy-related proteins.[8-9]

The lessened autophagic clearance in the RPE has been suggested to be related to the development and progression of AMD. This is supported by the detection of impaired

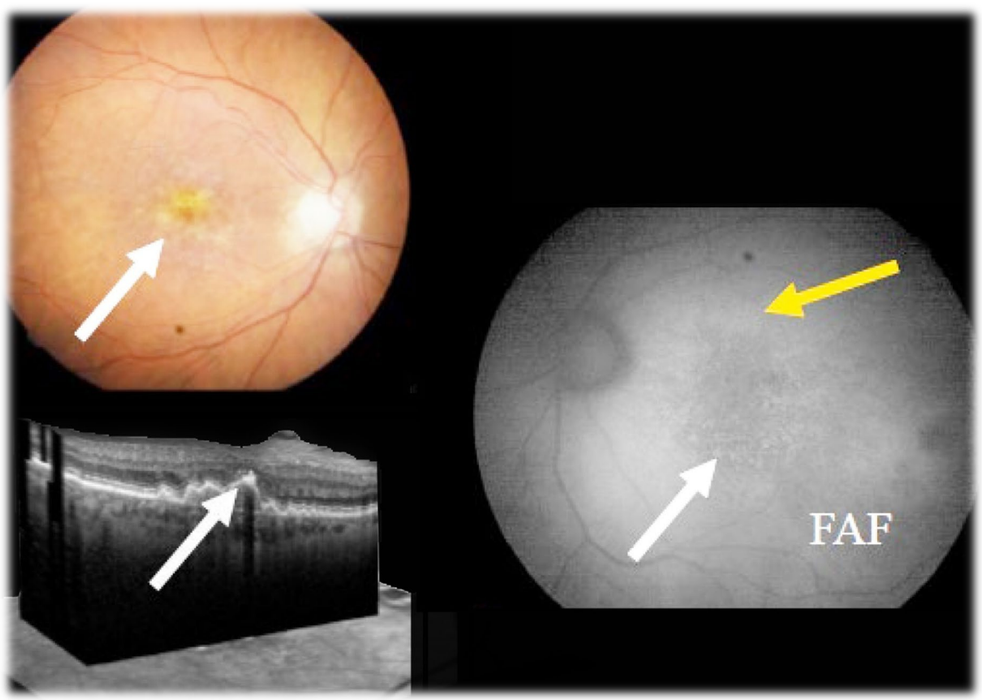


Figure 1. Lipofuscin and drusen accumulation as an indicator of disturbed proteolysis in AMD. Color fundus photograph, optical coherent tomography, and fundus autofluorescence (FAF) images of a dry AMD patient's retina. White arrows indicate drusen and the yellow arrow shows autofluorescent lipofuscin.

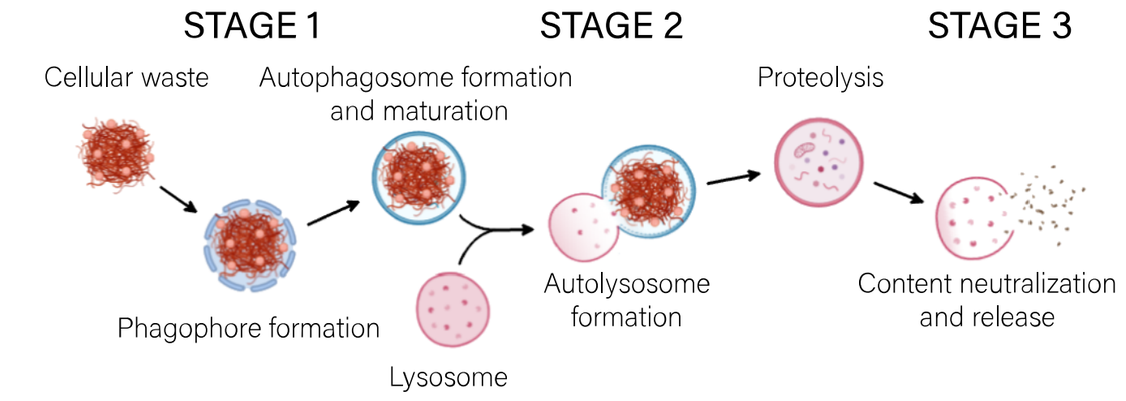


Figure 2. Stages of autophagy, simplified. The stages can be divided into 1) Induction/initiation and nucleation (waste engulfment and phagophore formation), 2) Membrane elongation (autophagosome formation), and 3) Fusion and degradation (autolysosome formation and proteolysis). Created with BioRender.com, with modifications.

cargo transport, disturbed waste clearance, and increased accumulation of lipofuscin, all of which are processes that increase oxidative stress in the RPE.[10-15] In the early phase of AMD, autophagy can counterbalance cellular stress-caused disturbances in the RPE, but in the later stage, reduced lysosomal activity leads to disease progression.[16-18]

MicroRNAs and their biogenesis

Epigenetic inheritance is currently described as phenotypic changes passed on to the next generation that cannot be detected via changes in the DNA base sequence of the genome.[19] Many types of non-coding RNA molecules are involved in controlling these changes. Possibly the best-known group of these, miRNAs, are especially important. Generally, miRNAs are described as short (about 17-25 nucleotides) single-stranded non-coding RNA segments.[20] They are critical in growth and development and are involved in a variety of other cellular processes.

The discovery of the first microRNA, lin-4, in 1993 by the Ambros group, led to a revolution in the field of molecular biology.[21] Since then, miRNAs have been detected in all kinds of animal model systems, and some have been shown to be highly conserved across species.[22] New miRNAs are still being discovered and their roles in gene regulation are still not fully understood. Abnormal expression of miRNAs is associated with many human diseases.[23]

MicroRNAs evolved to regulate the expression of messenger RNA (mRNA) transcripts, which are the intermediaries between genes and proteins. In that capacity, miRNAs are also involved in many diseases, including cardiovascular and neurodegenerative diseases and cancers. Currently, miRNAs are recognized as useful disease biomarkers and novel therapeutic targets.[24-26] It has been estimated that up to 60% of human mRNAs could be regulated by miRNAs.[27] Each miRNA can target many mRNAs (up to several hundred) and thus they have the capacity to regulate a wide range of biological targets.[28]

The biogenesis of miRNAs is a complicated, multi-step process (**Figure 3**). In short, it begins from the transcription of large primary miRNAs (pri-miRNAs) that get processed into considerably shorter precursor miRNAs, which eventually become mature, single-stranded miRNAs.[29] These bind in a sequence-specific manner to their target mRNAs. Once associated, miRNAs act either as a transcription or translation blocker/repressor,

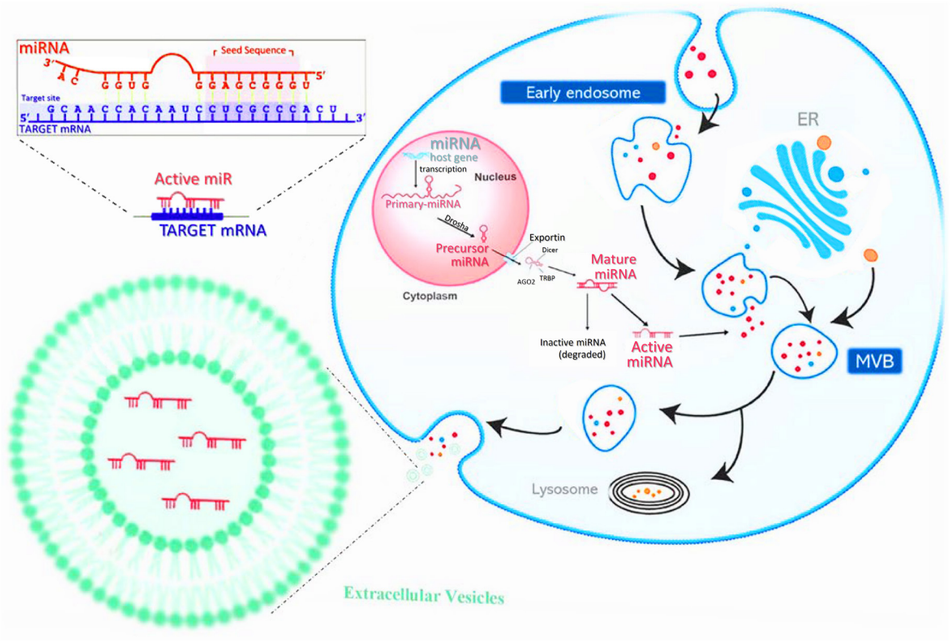


Figure 3. A schematic illustration of microRNA biogenesis, extracellular vesicle miRNA transfer, and miRNA binding method. The biogenesis of miRNA begins in the nucleus (red) with primary miRNA transcription. Afterwards, the primary miRNA is processed into a precursor miRNA by Drosha and DiGeorge syndrome critical region 8 (DGCR8) protein and is exported to the cytoplasm by Exportin 5. After enzymatic processing by Dicer, double-stranded, mature miRNAs are formed. By combining with Argonaute 2 (AGO2) and RNA-induced silencing complex (RISC), miRNAs can target mRNA transcripts, leading to mRNA degradation or repression. Active miRNAs can also be directly transferred to the recipient cells by microvesicles, which are shed from the cell's plasma membrane. On the other hand, mature miRNAs and some pre-miRNAs might be engulfed into multivesicular bodies (MVBs), which are generated via early-endosomal membrane invagination. These MVBs then dock onto the cell membrane and release positive exosomes into the extracellular space (including serum and other biological fluids). To exhibit their regulatory function, miRNAs bind to the target sites present in the 3′ untranslated region (UTR) of their target mRNAs. The seed sequence of a miRNA is defined as the first two to eight nucleotides at the beginning of their 5′ UTR. ER = endoplasmic reticulum, TRBP = transactivation response element RNA-binding protein.

or as a mediator of mRNA degradation.[30-34]

The specificity of miRNAs is regulated by a short, 2-8 base pairs-long seed sequence within each miRNA. As miRNAs do not completely match the base sequences of their target mRNAs, a perfectly matching seed sequence is a necessity to ensure the most stringent attachment to the target mRNA and the most effective suppression of the gene expression. As the effect of miRNAs comes out after matching complementally (base-specifically) with mRNAs, the base-pairing can be affected by the presence of single-nucleotide polymorphisms.[35] Polymorphism, in this case, refers to the natural population-level variation in how small parts of a gene, and therefore the resultant mRNA, may be coded.

MicroRNAs – the connection between AMD and autophagy

In total, hundreds of miRNAs have been found to be expressed in the retina.[36] For the readers of Oftalmolog, we have listed the effects and targets of key miRNAs, which have both been found to (1) be significantly up- or downregulated in AMD patients, and (2) have an effect on the autophagy machinery (**Figure 4**).

In the past decade, more and more data of miRNAs dysregulated in AMD has become available.[37-43] The numerous miRNAs affecting autophagy regulation have been discussed by Zhang and his co-authors.[44] The comprehensive compilation of the dysregulated miRNAs capable of regulating autophagy in AMD has been published this year by Hyttinen and others.[45]

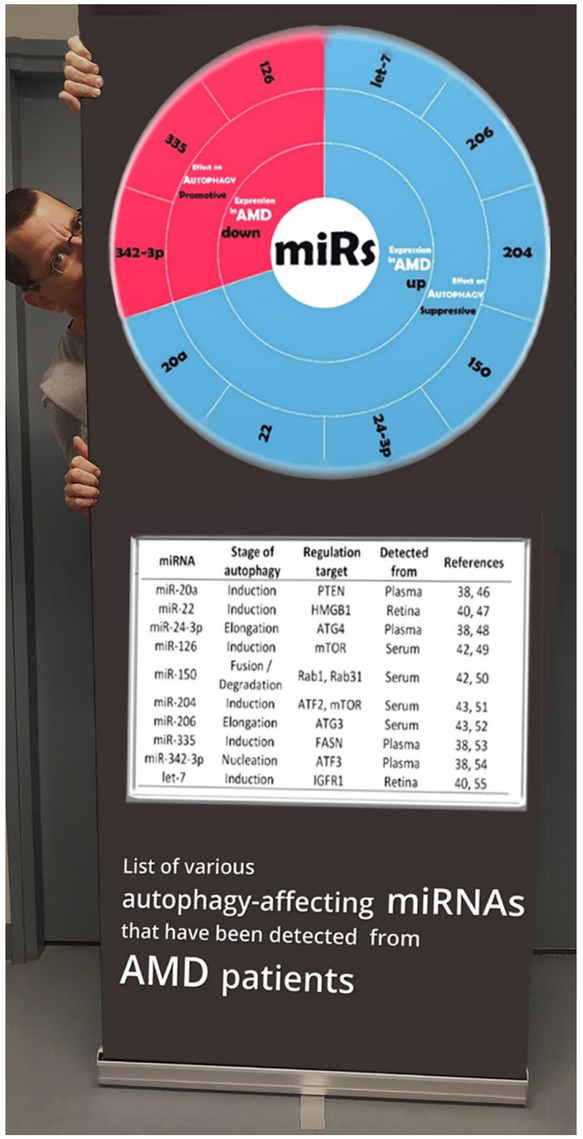


Figure 4. List of various autophagy-affecting miRNAs that have been detected from AMD patients. The study of microRNAs is a rapidly growing field of science as researchers discover new ones and uncover the importance of these small regulatory elements linked to AMD. Such interactions appear to provide fine-tuning effects on various cellular functions and contribute qualitatively to autophagy control. The ten miRNAs presented here are just a fraction of the miRNAs that are dysregulated in AMD. Targets of the molecular machinery governing autophagy are presented in the table. ATF = activating transcription factor, ATG = autophagy-related, FASN = fatty acid synthase, HMGB = high mobility group box, IGFR = insulin-like growth factor receptor, mTOR = mechanistic target of rapamycin, PTEN = phosphatase and tensin homolog, Rab = Ras-related protein.

Agomirs and antagomirs in therapy

Agomirs, or miRNA mimics, are synthetic miRNAs that can regulate mRNA function. They are designed based on the sequence of the mature miRNAs whose actions they are intended to mimic. The seed sequence must be identical to the natural miRNA's, but the rest of the agomir molecule may differ from the original. Typically, agomirs are modified to be more robust than natural miRNAs. They can also be tagged for cellular level monitoring.

On the other hand, antagomirs, or anti-miRNAs, have a complementary sequence to their corresponding miRNAs. Thus, they bind to and inhibit the expression and function of their target miRNAs, rather than mRNAs. These antagomirs can block the expression of the pri-miRNA of their target miRNA, inhibit the necessary processing steps to create the mature miRNA, or hinder the pairing of the mature miRNA with its target mRNA.[56]

According to the changes in the expression of autophagy-related miRNAs in AMD, or more specifically, when a pro-autophagic miRNA is downregulated or an anti-autophagic miRNA is upregulated, agomir or antagomir approaches could be considered in the therapy for the disease.

The expression changes of the miRNAs shown in the previous chapter could be modulated in the following manner:

- ***Upregulation of the targets of pro-autophagic miR-126, -335, and -342-3p,*** the levels of which are reported to decrease in AMD subjects, could be achieved by the agomirs of these miRNAs. In addition, the agomir of miR-126 could be useful against choroidal neovascularisation in wAMD due to its activity in maintaining vascular integrity.[57]

- ***A substantially larger number of upregulated anti-autophagic miRNAs have been detected in AMD subjects*** (e.g., miR-20a, -22, -24-3p, -150, -204, -206, and the members of the let-7 group). These could be targeted by their antagomirs to promote autophagy and counter AMD.

Extracellular vesicles and their ability as miRNA carriers

The chosen miRNAs could be carried to the retina by using extracellular vesicle (EV) -driven administration. In normal cellular operation, EVs are secreted from cells and can carry bioactive molecules, such as genetic material, within them. These can then be delivered to neighboring cells or more distant targets. They effectively enable intercellular communication.[58-59]

In drug manufacturing, the chosen therapeutic material, like miRNAs, can be directly incorporated into EVs in vitro. Alternatively, they can be transfected into suitable cells, such as mesenchymal stem cells, where they can become encapsulated within EVs before they are released outside the cell, then extracted, and used as carriers of their contents (**Figure 5**). In addition, miRNAs are found to be stable when packed in EVs.[60]

In the case of the eye, and especially the retina, the most effective means of therapy could be injecting EVs into the vitreous humour. From there, miRNAs can reach the bottom of the eye and target the RPE layer there.[61] This is further aided by the excellent capacity of the RPE to efficiently phagocytize external particles, as they naturally phagocytize the outer segments of photoreceptors as part of photoreceptor maintenance.[62]

Extracellular vesicle miRNA-therapy models have recently been reported against other diseases. As an example, the intravitreal administration of miR-126-containing extracellular vesicles in rats reduced hyperglycaemia-induced retinal inflammation by targeting high mobility group box 1 mRNA.[63]

Conclusions & perspectives

As the weakening of autophagy is thought to be a cause of AMD progression, the strengthening of it might help relieve the effects of this harmful disease. This could be achieved through miRNAs (**Figure 6**). The progress in epigenomics, i.e. the study of inheritable changes in gene expression that do not involve changes in the DNA sequence, as well as the evolving understanding of the structure,

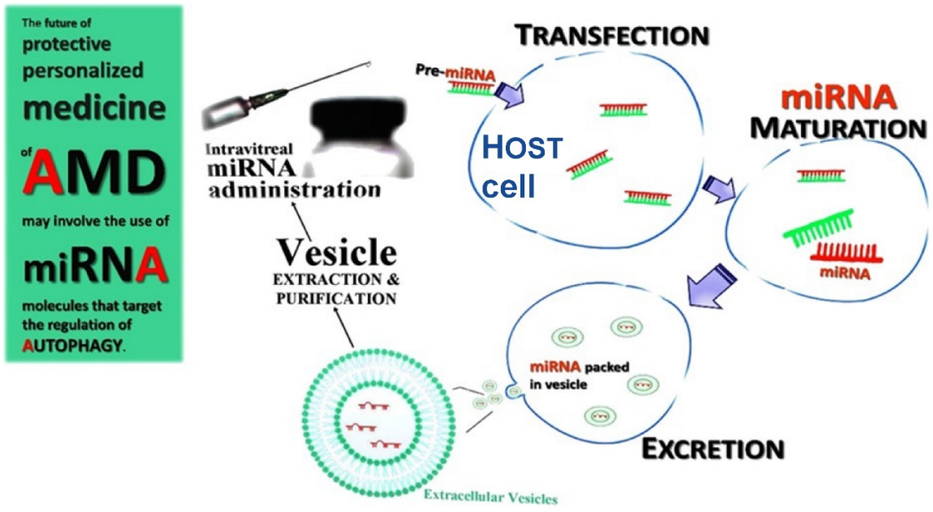
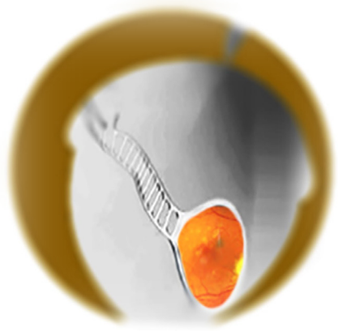


Figure 5. A schematic illustration of microRNA biogenesis and extracellular vesicle microRNA transfer. Modified from Hyttinen et al., Ageing Res Rev. 2021;67:101260.



AMD & microRNAs in a nutshell

Nuts about the impact of **microRNAs** in **AMD**

Strengthening of **autophagy** might **help relieve** the effects of **AMD.**

Many microRNAs play important roles **in fine regulation of autophagy.**

Advancing our **knowledge** about the
(1) miRNA target identification,
(2) miRNA delivery, and
(3) their specificity in **AMD** is **central** to **harnessing** their **potential** as **therapeutic agents** to counteract effects of **AMD** and visual impairment.

Figure 6. Take-home message on microRNAs in AMD. Modified from Hyttinen et al., Ageing Res Rev. 2021;67:101260.

function, and mutual interactions between different miRNAs may lead to them becoming more easily utilizable as therapeutic targets for AMD and other diseases.

Treatment using miRNAs might provide the possibility of targeted personal therapy in the future. As a simplified example, the expressions of AMD-connected miRNAs could be measured in a patient and, if dysregulated miRNAs are found, the applicable miRNA therapy profile could be chosen, followed by administering specific miRNAs to the eye.[64]

Before these kinds of therapies can succeed, several issues must be resolved. These can be grouped into:

(1) target identification,
(2) specificity, and
(3) delivery.

As miRNAs tend to target many mRNAs, discovering as many of these interactions as possible is of great importance. Once the targets are known, selecting or creating sufficiently specifically binding miRNAs becomes the next crucial step. In the case of multi-factorial diseases such as AMD, delivery of insufficiently researched miRNAs could potentially disturb the expressions of other essential mRNAs. In the worst case, this might even accelerate the progression of AMD. Delivery is an obstacle to which EV-derived administration could be a good solution. The natural delivery system of EVs offers enormous potential as a delivery mechanism, particularly in conjunction with suitable targeting ligands on their surface. This might allow for the creation of a bio-compatible, personalized delivery system with added specificity to damaged RPE cells.

Translational and clinical research aimed at efficiently delivering miRNAs into the RPE might enhance the effectiveness of future therapeutic interventions for AMD. With increasingly well-characterized expressions and functions within RPE cells, miRNAs are some of the most promising therapeutic

treatments. However, further deep research is still needed in this field. As AMD will become an ever more pervasive disease with the aging of the human population, every possible means should be investigated on the chance that they eventually would provide an effective cure against this highly detrimental disease.[45]

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REFERENCES

1. Ambati J, Fowler BJ. Mechanisms of age-related macular degeneration. Neuron 2012;75:26-39.
2. Ding X, Patel M, Chan CC. Molecular pathology of age-related macular degeneration. Prog Retin Eye Res. 2009;28:1-18.
3. Hernández-Zimbrón LF, Zamora-Alvarado R, Ochoa-De la Paz L, Velez-Montoya R, Zenteno, E, Gulias-Cañizo R, Quiroz-Mercado H, Gonzales-Salinas R. Age-related macular degeneration: New paradigms for treatment and management of AMD. Oxid Med Cell Longev. 2018;2018:8374647.
4. Kaarniranta K, Uusitalo H, Blasiak J, Felszeghy S, Kannan R, Kauppinen A, Salminen A, Sinha D, Ferrington D. Mechanisms of mitochondrial dysfunction and their impact on age-related macular degeneration. Prog Retin Eye Res. 2020;79:100858.
5. Mizushima N. Autophagy: Process and function. Genes Dev. 2007;21:2861-2873.
6. Kaarniranta K, Tokarz P, Koskela A, Paterno J, Blasiak J. Autophagy regulates death of retinal pigment epithelium cells in age-related macular degeneration. Cell Biol Toxicol. 2017;33:113-128.
7. Rezzani R, Stacchiotti A, Rodella LF. Morphological and biochemical studies on aging and autophagy. Ageing Res Rev. 2012;11:10-31.
8. Lapierre LR, Kumsta C, Sandri M, Ballabio A, Hansen M. Transcriptional and epigenetic regulation of autophagy in aging. Autophagy 2015;11:867-880.
9. Salminen A, Kaarniranta K. AMP-activated protein kinase (AMPK) controls the aging process via an integrated signaling network. Ageing Res Rev. 2012;11:230-241.
10. Abokyi S, To CH, Lam TT, Tse DY. Central role of oxidative stress in age-related macular degeneration: Evidence from a review of the molecular mechanisms and animal models. Oxid Med Cell Longev. 2020;2020:7901270.
11. Bales KL, Gross AK. Aberrant protein trafficking in retinal degenerations: The initial phase of retinal remodeling. Exp Eye Res. 2016;150:71-80.
12. Blasiak J, Petrovski G, Veréb Z, Facskó A, Kaarniranta K. Oxidative stress, hypoxia, and autophagy in the neovascular processes of age-related macular degeneration. Biomed Res Int. 2014;2014:768026.
13. Boya P, Esteban-Martínez L, Serrano-Puebla A, Gómez-Sintes R, Villarejo-Zori B. Autophagy in the eye: Development, degeneration, and aging. Prog Retin Eye Res. 2016;55:206-245.
14. Golestaneh N, Chu Y, Xoa YY, Stoleru GL, Theos AC. Dysfunctional autophagy in RPE, a contributing factor in age-related macular degeneration. Cell Death Dis. 2017;8:e2537.
15. Keeling E, Lotery AJ, Tumbarell, DA, Ratnayaka JA. Impaired cargo clearance in the retinal pigment epithelium (RPE) underlies irreversible blinding diseases. Cells 2018;7:E16.
16. Felszeghy S, Viiri J, Paterno JJ, Hyttinen JMT, Koskela A, Chen M, Leinonen HO, Tanila H, Kivinen N, Koistinen A, Toropainen E, Smedowski A, Reinisalo M et al. Loss of NRF-2- and PGC-1α genes leads to retinal pigment epithelium damage resembling dry age-related macular degeneration. Redox Biol. 2019;20:1-12.
17. Ferrington DA, Sinha D, Kaarniranta K. Defects in retinal pigment epithelial cell proteolysis and the pathology associated with age-related macular degeneration. Prog Retin Eye Res. 2016;51:69-89.
18. Viiri J, Amadio M, Marchesi N, Hyttinen JMT, Kivinen N, Sironen R, Rilla K, Akhtar S, Provenzani A, D'Agostino VG, Govoni S, Pascale A, Agostini H et al. Autophagy activation clears ELAVL1/HuR-mediated accumulation of SQSTM1/p62 during proteasomal inhibition in human retinal pigment epithelial cells. PLoS One 2013;8:e69563.
19. Riggs AD, Martienssen RA, Russo VEA. Introduction. In: Russo VEA et al. (eds.), Epigenetic Mechanisms of Gene Regulation. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA,1996:1-4.
20. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet. 2004;5:522-531.
21. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993;75:843-854.
22. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, et al. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature 200;408:86-89.
23. Paul P, Chakraborty A, Sarkar D, Langthasa M, Rahman M, Bari M, et al. Interplay between miRNAs and human diseases. J Cell Physiol. 2018;233:2007-2018.
24. Bartel DP. Metazoan microRNAs. Cell 2018;173:20-51.
25. Ebert MS, Sharp PA. Roles for microRNAs in conferring robustness to biological processes. Cell 2012;149:515-524.
26. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov. 2017;16:203-222.
27. Friedman RC, Fahr KKH, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of miRNAs. Genome Res. 2009;19:92-105.
28. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 2005;433:769-773.
29. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol. 2014;15:509-524.
30. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281-297.
31. Griffiths-Jones, S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res. 2006;34 (Database issue):D140-144.
32. Kuo WT, Yu SY, Li SC, Lam HC, Chang HT, Chen WS, Yeh CY, Hung SF, Liu TC, Wu T, Yu CC, Tsai KW. MicroRNA-324 in human cancer: miR-324-5p and miR-324-3p have distinct biological functions in human cancer. Anticancer Res. 2016;36:5189-5196.
33. Li SC, Liao YL, Ho MR, Tsai KW, Lai CH, Lin WC. miRNA arm selection and isomiR distribution in gastric cancer. BMC Genomics 2012;13 (Suppl. 1):S13.
34. Wei JW, Huang K, Yang C, Kang CS. Non-coding RNAs as regulators in epigenetics (Review). Oncology Rep. 2017;36:3-9.
35. Wang X. Composition of seed sequence is a major determinant of microRNA targeting patterns. Bioinformatics 2014;30:1377-1783.
36. Soundara Pandi SP, Chen M, Guduric-Fuchs J, Xu H, Simpson DA. Extremely complex populations of small RNAs in the mouse retina and RPE/choroid. Invest Ophthalmol Vis Sci. 2013;54:8140-8151.
37. Blasiak J, Watala C, Tuuminen R, Kivinen N, Koskela A, Uusitalo-Järvinen H, Tuulonen A, Winiarczyk M, Mackiewicz J, Zmorzyński S, Filip A, Kaarniranta K. Expression of VEGFA-regulating miRNAs and mortality in wet AMD. J Cell Mol Med. 2019;23:8464-8471.
38. Ertekin S, Yıldırım O, Dinç E, Ayaz L, Fidancı SB, Tamer L. Evaluation of circulating miRNAs in wet age-related macular degeneration. Mol Vis. 2014;20:1057-1066.
39. Grassmann F, Schoenberger PG, Brandl C, Schick T, Hasler D, Meister G, Fleckenstein M, Lindner M, Helbig H, Fauser S, Weber BH. A circulating microRNA profile is associated with late-stage neovascular age-related macular degeneration. PLoS One 2014;9:e107461.
40. Lukiw WJ, Surjyadipta B, Dua P, Alexandrov PN. Common micro RNAs (miRNAs) target complement factor H (CFH) regulation in Alzheimer's disease (AD) and in age-related macular degeneration (AMD). Int J Bichem Mol Biol. 2012;3:105-116.
41. Ménard C, Rezende FA, Miloudi K, Wilson A, Tétreault N, Hardy P, SanGiovanni JP, De Guire V, Sapieha P. MicroRNA signatures in vitreous humour and plasma of patients with exudative AMD. Oncotarget 2016;7:19171-19184.
42. Romano GL, Platania CBM, Drago F, Salomone S, Ragusa M, Barbagallo C, Di Pietro C, Purrello M, Reibaldi M, Avitabile T, Longo A, Bucolo C. Retinal and circulating miRNAs in age-related macular degeneration: An in vivo animal and human study. Front Pharmacol. 2017;8:168.
43. Szemraj M, Bielecka-Kowalska A, Oszajca K, Krajewska M, Goś R, Jurowski P, Kowalski M, Szemraj J. Serum microRNAs as potential biomarkers of AMD. Med Sci Monit. 2015;21:2734-2742.
44. Zhang J, Wang P, Wan L, Xu S, Pang D. The emergence of noncoding RNAs as Heracles in autophagy. Autophagy 2017;13:1004-1024.
45. Hyttinen JMT, Blasiak J, Felszeghy S, Kaarniranta K. MicroRNAs in the regulation of autophagy and their possible use in age-related macular degeneration therapy. Ageing Res Rev. 2021;67:101260.
46. Gong Y, Xu W, Chen Y, Liu Y, Yang Y, Wang B, Lu Z, Lin HC, Zhou X, Zhou X. miR-20a-5p regulates pulmonary surfactant gene expression in alveolar type II cells. J Cell Mol Med. 2019;23:7664-7672.
47. Guo S, Bai R, Liu W, Zhao A, Zhao Z, Wang Y, Wang Y, Zhao W, Wang W. miR-22 inhibits osteosarcoma cell proliferation and migration by targeting HMGB1 and inhibiting HMGB1-mediated autophagy. Tumour Biol. 2014;35:7025-7034.
48. Pan B, Chen Y, Song H, Xu Y, Wang R, Chen L. Mir-24-3p downregulation contributes to VP16-DDP resistance in small-cell lung cancer by targeting ATG4. Oncotarget 2014;6:317-331.
49. Tang F, Yang TL. MicroRNA-126 alleviates endothelial cells injury in atherosclerosis by restoring autophagic flux via inhibiting of PI3K/Akt/mTOR pathway. Biochem Biophys Res Commun. 2018;495:1482-1489.
50. Liu X, Fu B, Chen D, Hong Q, Li J, Bai X, Chen X. miR-184 and miR-150 promote renal glomerular mesangial cell aging by targeting Rab1a and Rab31. Exp Cell Res. 2015;336:192-203.
51. Li N, Guo X, Liu L, Wang L, Cheng, R. Molecular mechanism of miR-204 regulates proliferation, apoptosis and autophagy of cervical cancer cells by targeting ATF2. Artif Cells Nanomed Biotechnol. 2019;47:2529-2535.
52. Kong F, Jin J, Lv X, Han Y, Liang X, Gao Y, Duan X. Long noncoding RNA RMRP upregulation aggravates myocardial ischemia-reperfusion injury by sponging miR-206 to target ATG3 expression. Biomed Pharmacother. 2019;109:716-725.
53. Gao XL, Li JQ, Dong YT, Cheng EJ, Gong JN, Qin YL, Huang YQ, Yang JJ, Wang SJ, An DD. Upregulation of microRNA-335-5p reduces inflammatory responses by inhibiting FASN through the activation of AMPK/ULK1 signaling pathway in a septic mouse model. Cytokine 2018;110:466-478.
54. Han Y, Zhang K, Hong Y, Wang J, Liu Q, Zhang Z, Xia H, Tang Y, Li T, Li L, Xue Y, Hong W. miR-342-3p promotes osteogenic differentiation via targeting ATF3. FEBS Lett. 2018;592:4051-4065.
55. Hou C, Zhu M, Sun M, Lin Y. MicroRNA let-7i induced autophagy to protect T cell from apoptosis by targeting IGF1R. Biochem Biophys Res Commun. 2014;453:728-734.
56. Nguyen DD, Chang S. Development of novel therapeutic agents by inhibition of oncogenic microRNAs. Int J Mol Sci. 2017;19:E65.
57. Zhao F, Anderson C, Karnes S, Zhou Q, Ma J, Jin ZG, Bhattacharjee PS, Wang S. Expression, regulation and function of miR-126 in the mouse choroid vasculature. Exp Eye Res. 2018;170:169-176.
58. Camussi G, Deregibus, MC, Cantaluppi V. Role of stem-cell-derived microvesicles in the paracrine action of stem cells. Biochem Soc Transact. 2013;41:283-287.
59. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biological function and clinical potential. Cell Biosci. 2019;9:19.
60. Ge Q, Zhou Y, Lu J, Bai Y, Xie X, Lu Z. miRNA in plasma exosome is stable under different storage conditions. Molecules 2014;19:1568-1575.
61. Mead B, Tomarev S. Extracellular vesicle therapy for retinal diseases. Prog Retin Eye Res. 2020;79:100849.
62. Strauss O. The retinal pigment epithelium in visual function. Physiol Rev. 2005;85:845-881.
63. Zhang W, Wang Y, Kong Y. Exosomes derived from mesenchymal stem cells modulate mir-126 to ameliorate hyperglycemia-induced retinal inflammation via targeting HMGB1. Invest Ophthalmol Vis Sci. 2019;60:294-303.
64. Paterno JJ, Koskela A, Hyttinen JMT, Vattulainen E, Synowiec E, Tuuminen R, Watala C, Blasiak J, Kaarniranta K. Autophagy Genes for Wet Age-Related Macular Degeneration in a Finnish Case-Control Study. Genes (Basel). 2020;11(11):1318.

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