LIFE SAFETY AND SECURITY

open access

Original Paper

Manuscript

A PILOT ANALYSIS OF SMALL NUCLEOLAR RNA EDITOME OF OXIDATIVE STRESSED RETINAL EPITHELIAL CELLS REVEALED A POSSIBLE ROLE FOR DYSREGULATED NEUROTRANSMISSION IN INHERITED RETINAL DISEASES

Luigi Donato[1,2], Concetta Scimone[1,2], Simona Alibrandi[1,2,3], Domenico Mordà[1,2], Alessandra Costa[2,4], Fabiana Nicita[1,2], Rosalia D'Angelo[1,2], Antonina Sidoti[1,2]

[1]Department of Biomedical and Dental Sciences and Morphofunctional Images, Division of Medical Biotechnologies and Preventive Medicine, University of Messina, Messina, Italy

[2]Department of Biomolecular strategies, genetics and cutting-edge therapies, I.E.ME.S.T., Palermo, Italy

[3]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

[4] Department of Economics, University of Messina, Messina, Italy

CORRESPONDENCE: Luigi Donato
e-mail: luigidonato@iemest.eu
Phone number: +390902213136

Received: December 9th, 2022
Revised: December 18th, 2022
Accepted: December 19th, 2022

Abstract

The growing knowledge on non-coding RNAs (ncRNAs) suggests that a significant number of transcriptional and posttranscriptional regulative mechanisms in eukaryotes could represent the key to improve our knowledge on many diseases etiopathogenesis, like inherited retinal diseases (IRDs). Among ncRNAs, small nucleolar RNAs (snoRNAs) is still an unexplored world. We investigated the posttranscriptional RNA editing landscape of human retinal pigment epithelium cells (RPE) exposed to the oxidant agent N-retinylidene-N-retinyl ethanolamine (A2E) for 3 h and 6 h, focusing on snoRNA host and target genes. Using a transcriptomic approach, refined with a specific multialgorithm pipeline, 9 snoRNA editing sites within related host genes were identified among all samples. Such genes resulted involved in several biochemical pathways linked to retinal neurotransmission. SnoRNA epitranscriptome analysis of oxidative stress induced RPE cells suggested that such ncRNAs could play a relevant role in IRD etiopathogenesis, regulating pathways directly or indirectly related to the considered disease.

Keywords

Retina; RNA-Seq; snoRNA; ncRNA; Epitranscriptome.

Introduction

Posttranscriptional RNA modifications (PTMs) provide extending function and diversity of transcripts. One of the most fascinating PTMs is RNA editing, that increases biologically relevant diversity of transcripts or protein isoforms [1]. RNA editing is a recently discovered mechanism that modifies the RNA sequence itself without changing its genomic DNA (gDNA) through nucleotide deletion, insertion, and substitution [2]. Presently, it is known that such modifications may determine transcriptome plasticity and diversity along with changing amino acids, affecting RNA stability, modulating the nuclear retention of RNAs, and impacting alternative splicing (AS) [3]. RNA editing by base conversion can induce different biological effects depending on RNA type (mRNA or non-coding RNA), region (5′ or 3′ untranslated region (UTR)), coding sequence (CDS) or intron involved in the modification [4]. Consequently, dysregulation of the RNA editing process has been reported to be associated with several human neurodegenerative and psychiatric disorders, such as autism spectrum disorder and amyotrophic lateral sclerosis (ALS) [5]. Furthermore, recent discoveries on non-coding RNAs (ncRNAs) suggest that a huge number of regulative mechanisms of transcription, maturation (e. g. splicing, [6]) and translation in eukaryotes could represent the key to improve our knowledge on many diseases etiopathogenesis [7]. In eukaryotes, the ribosomal RNA (rRNA) requires several site-specific post-transcriptional nucleotide modifications, most relevant of which are represented by 2′-O-methylation and pseudouridylation [8]. Both processes occur in the nucleolus and involve a huge number of small nucleolar RNAs (snoRNAs), made of 60–300 nucleotides, as well as multicomponent complexes, constituted by small nucleolar ribonucleoprotein (snoRNP) [9]. SnoRNAs can be divided into two classes which possess distinctive sequence elements: the highly conserved box C/D snoRNAs promote the 2′-O-methylation of specific sites on rRNA, thanks to the methyltransferase fibrillarin [10]. The second group, represented by the box H/ACA snoRNAs, guide pseudouridine modifications at specific sites on rRNA, with the help of the pseudouridine synthase dyskeri and several additional proteins like GAR1 and NOP10 [11]. Interestingly, there is a growing evidence that guide snoRNA targets are not limited to rRNA, as evidenced by identification of many “orphan snoRNAs” with already unknown target RNAs [12]. Even if the exact function of different types of rRNA modifications is not totally clear, there is now a

mounting hypothesis that the machinery needed for site-specific rRNA modifications is required for normal development and is altered in numerous human pathologies, as cancer, neurodegenerative and genetic diseases [13]. Many evidences support the role of snoRNAs in normal retinal development and functions, but exact mechanisms are still unknown [14]. Therefore, an improved knowledge of human retina snoRNAs, especially of patients affected by retinal disease, could lead to better understanding the unappreciated functional role of snoRNAs related to physiopathology of this tissue. Today the link between RNA editing events and snoRNAs was never explored. Such a complex scenario has highlighted the need for novel technologies to investigate the effects of RNA editing events, and the advancement of computational methods has enabled the elucidation of many RNA editing sites in humans [15]. Today, RNA-Seq is the gold standard approach to discover RNA editing candidates in whole eukaryotic genomes [16]. In the present work, for the first time we describe the RNA editing event occurring in snoRNAs detected within retinal pigment epithelium (RPE) cells in a physiological condition versus exposition to the oxidant agent N-retinylidene-N-retinyl ethanolamine (A2E), a toxic bis-retinoid capable of reproducing an oxidative stressed microenvironment typical of many retinal dystrophies [17, 18]. H-RPE cells play a critical role in visual function and photoreceptor viability and, as highlighted in our previously published works, treatment with A2E may determine significant differences in gene expression and splicing events, involving many biological pathways recently related to retinal degeneration [19, 20].

Materials and Methods

Cell Culture and Oxidative Stress Induction

Human RPE-derived cells (H-RPE—Human Retinal Pigment Epithelial Cells, Clonetics™, Lonza, Basel, Switzerland, cat. n°: 00194987) were cultivated following previously described protocols [21]. Cells are cryopreserved, primary cells that are packaged at passage 2 and contain ≥500,000 cells per vial. H-RPE cells were then plated into 96-well plates (4×10^4 cells/well) and cultivated for 24h to reach confluence before A2E (AptaBio, Yongin, Korea) addition, in a final concentration of 20 µM for 6h and following rinsing with medium. Control cell groups were incubated without A2E. Confluent cultures were transferred to PBS supplemented with Mg^{2+}, Ca^{2+} and glucose, before exposure to blue light emitted by a tungsten-halogen source (470 ± 20 nm; 0.4 mW/mm^2) for 30 min to induce phototoxicity of A2E and subsequent incubation at 37 °C, as already described by confirmed protocols [22-24]. The first 3 generations of subcultured RPE

cells were used in this experiment. Finally, cell viability was assessed by MTT assay, following a previously described protocol [21].

Bulk RNA Sequencing

Whole RNA was isolated by TRIzol™ Reagent (Invitrogen™, ThermoFisher Scientific, Waltham, MA, USA) following manufacturer's protocol, and quantified at Qubit 2.0 fluorimeter. The RNA-Seq samples consisted of 3 factor groups, represented by H-RPE cells, before treatment with A2E and at two following time points of 3 h and 6 h, chosen on the basis of our precedent experiences [21]. Libraries were generated using 1 µg of total RNA by the TruSeq Stranded Total RNA Sample Prep Kit with Ribo-Zero H/M/R (Illumina, San Diego, CA, USA), according to manufacturer's protocols. Sequencing runs were performed on a HiSeq 2500 Sequencer (Illumina, San Diego, CA, USA), using the HiSeq SBS Kit v4 (Illumina, San Diego, CA, USA). The experiment was repeated three times. Obtained raw sequences were filtered to remove low-quality reads (average per base Phred score <30) and adaptor sequences. The quality of analyzed data was checked using FastQC (v.0.11.9) (Babraham Institute, Cambridge, UK, https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and QualiMap (v.2.2.1) [25], while trimming was realized by Trimmomatic (v.0.39) (Max Planck Institute of Molecular Plant Physiology, Golm, Germany). The filtered data was, then, analyzed by CLC Genomics Workbench v.22.0 (https://digitalinsights.qiagen.com/products-overview/analysis-and-visualization/qiagen-clc-genomics-workbench/), using *Homo sapiens* genome hg38 and Ensembl RNA database v.105 as references. RNA – Seq analysis was conducted using the following settings: quality trim limit = 0.02, ambiguity trim maximum value=2. Map to annotated reference: minimum length fraction and minimum similarity fraction=0.7, maximum number of hits/read=2, type of organism = eukaryote, paired settings = default.

snoRNA Editing Event Identification and Comparison between Treated and Control Samples

Once the alignment phase was concluded, the RNA editing detection step was performed. The analytic pipeline started with variant calling using the fixed ploidy variant detection in the CLC Genomics Workbench suite, reporting variants with >95% probability. Furthermore, to rescue low-confidence editing events for which editing was not detected de novo, we compared identified editing sites with ones annotated in the RADAR [26] and REDIportal [26] databases, using the REDItools [27]. *De novo* editing sites were

filtered according to the Bonferroni-adjusted *p*-value and only those showing a *p*-value of < 0.05 were selected for downstream analysis. Using the BamDeal toolkit (https://github.com/BGI-shenzhen/BamDeal), identified RNA editing events were filtered on the basis of high-confidence, defined by the following criteria: (1) an editing site showing read coverage of >100 required at least 3 mutant reads to be considered edited with high confidence; (2) sites with 20–99 reads of coverage needed at least 2 mutant reads; (3) for sites with <20 reads of coverage, only 1 mutant read was required to consider them as high confidence. The editing level of each site was calculated as Gs/As + Gs. The overall editing level of each sample was calculated as the number of Gs divided by the total number of As + Gs at all editing sites. Found editing events were, then, compared after dividing them on the bases of snoRNA involved host genes.: (1) genes edited in both 3 h and 6 h treated samples but showing different editing events and frequency; (2) genes only edited in 3 h treated samples; (3) genes only edited in 6 h treated samples. For each editing site, editing ratio between treated samples and/or control was calculated. Frequencies of editing events were calculated by IBM SPSS Statistics 26.0 software [28].

2.4. snoRNA Target Identification and Host Genes Pathway Analysis

Finally, previously filtered snoRNAs were firstly analyzed by the experimentally validated snoRNAs database snoDB [29], in order to define specific targets and host genes. Once obtained them, a pathway analysis of selected host genes was performed by Cytoscape and its plug in ClueGO [30].

Results

Sequencing analysis and mapping statistics

RNA sequencing carried out on Illumina NovaSeq 6000 (Illumina, San Diego, CA, USA) yielded a total of approximately 96 million quality reads (mean mapping quality = 29) with a percentage of 68% uniquely mapped. About 16,200 genes and 70,000 transcripts were identified out of 60,609 and 227,462 reference counterparts, respectively, considering the whole human transcriptome. The annotated reference assembly v.40 (GRCh38.p13) was downloaded from GeneCode FTP server (ftp://ftp.ebi.ac.uk/pub/databases/gencode/Gencode_human/). All previous mapping statistics were based on average values calculated for all three replicates in each time point.

Editing Site Comparison between Control and Treated RPE Cells highlighted events in 9 snoRNA host genes

Detected editing events were found within 9 genes encoding small nucleolar RNAs. Most editing events that occurred during the whole experimental period, especially after 3 h and 6 h from treatment. Specific host/target genes for 5 of 9 investigated snoRNAs, divided into 2 H/ACA box snoRNAs (SNORA63B and SNORA17B) and into 7 C/D box snoRNAs (SNORD46, SNORD117, SNORD50B, SNORD141A, SNORD1B, SNORD3A, SNORD118) were found. For the others, instead, host/target genes resulted still unknown. Very interesting, the common edited genes were mainly involved in the homeostasis of neuronal system, as suggested by the macro – pathways "Neurotransmitter receptors and postsynaptic signal transmission", "Transmission across Chemical Synapses" and other similar ones emerged from pathway analysis of host/target genes. Details on edited gene pathway enrichment are available in Table 1.

GENE SYMBOL		*SNORD46*	*SNORA63B*	*SNORD117*	*SNORD50B*	*SNORD141A*	*SNORA17B*	*SNORD1B*	*SNORD3A*	*SNORD118*
Consequence		downstream	transcript	upstream	upstream	transcript	downstream	upstream	transcript	upstream
Chromosome		1	3	6	6	6	9	17	17	17
Position		44777998	186786359	31538847	85678170	73518351	136724914	76559024	19188159	8173460
Reference		A	T	T	G	T	C	T	C	C
Strand		2	2	2	2	2	2	2	2	2
Box		C/D	H/ACA	C/D	C/D	C/D	H/ACA	C/D	C/D	C/D
Target RNA		28S rRNA	Unknown	Unknown	Nonfunct.	Unknown	Unknown	28S rRNA	Unknown	28S rRNA
Organization		Intronic	Unknown	Intronic	Intronic	Unknown	Unknown	Intronic	Unknown	Intronic
Host Locus		RPS8	Unknown	BAT1	Host Gen.5	Unknown	Unknown	BC042949	Unknown	CHRFAM7A
CTRL	Coverage-q30	15	3	26	23	11	41	9	3	3
	MeanQ	31	32	31	32	31	32	32	31	32
	BaseCount[A,C,G,T]	[0, 0, 0, 2]	[0, 2, 0, 3]	[0, 2, 0, 10]	[0, 0, 1, 14]	[0, 3, 0, 5]	[0, 25, 17, 0]	[0, 7, 0, 0]	[0, 0, 0, 1]	[0, 0, 0, 3]
	AllSubs	AT	TC	TC	GT	TC	CG	TC	CT	CT
	Frequency	1,00	0,63	0,26	0,82	0,45	0,35	1,00	0,78	0,61
3h	Coverage-q30	14	/	33	17	4	24	9	/	/
	MeanQ	31	/	32	34	32	33	33	/	/
	BaseCount[A,C,G,T]	[0, 0, 0, 8]	/	[0, 5, 0, 15]	[0, 0, 0, 14]	[0, 4, 0, 2]	[0, 12, 10, 0]	[0, 9, 0, 0]	/	/
	AllSubs	AT	/	TC	GT	TC	CG	TC	/	/
	Frequency	1,00	/	0,35	1,00	0,58	0,42	1,00	/	/
6h	Coverage-q30	12	/	25	15	/	26	10	/	/
	MeanQ	32	/	32	33	/	32	32	/	/
	BaseCount[A,C,G,T]	[0, 0, 0, 16]	/	[1, 3, 0, 18]	[0, 0, 0, 19]	/	[0, 17, 7, 0]	[0, 6, 0, 0]	/	/
	AllSubs	AT	/	TC TA	GT	/	CG	TC	/	/
	Frequency	0,89	/	0,26	0,89	/	0,48	1,00	/	/

Table 1. Distribution and features of identified known editing sites across time points.

Discussion

Eukaryotic organisms are characterized by complex transcriptomes whose regulation is critical for all cellular processes and homeostasis [31]. The dynamicity of transcriptomes is based on greatly modulated posttranscriptional mechanisms, such as alternative splicing and RNA modifications [32]. RNA editing is taking on a pivotal role in promoting transcriptome diversity and fine-tuning gene expression [33]. With the growing adoption of genome re-sequencing and RNA-Seq technologies, a considerable number of RNA editing sites in the genome of several animal species, Homo sapiens included, have been identified [33]. Currently, RNA editing in physiological and pathological human retinas remains quite unknown, and it could become clinically relevant in many diseases, such as age-related macular degeneration (AMD), diabetic retinopathy, and other retinal degenerations [34, 35]. Additionally, in the wide world of inherited retinal diseases (IRDs), little is known about regulative non – coding RNAs involvement [36, 37]. The only validated knowledge regards miRNA role [38], which opened new frontiers about 3' UTR and intron variants involvement into etiopathogenesis of such pathologies [39], giving ncRNAs the status of most promising targets of experimental therapies [40]. Among them, Retinitis pigmentosa, an ocular disease with very heterogeneous phenotypes, shows unusually complex molecular genetic causes, most of which still unknown [41]. In the present study, we carried out a comprehensive profiling of RPE cells treated with A2E during a follow-up of two time points (3 h and 6 h) after exposure and compared them to untreated time zero controls. Using strand-specific RNA-Seq datasets with high sequencing depth and coverage, together with a complex multi-alignment data analysis pipeline, we were able to capture unprecedented editing events with low editing levels and in low depth regions, focusing on snoRNA changes during RPE cell death induced by the toxic effects of A2E. We detected 9 already annotated and de novo RNA editing sites throughout all time-related samples, decreasing from basal conditions to 6h from A2E exposure, and suggesting that the induced oxidative stress could heavily impair RNA editing activity. Oxidative stress plays a critical role in the etiopathogenesis of IRDs [42], especially targeting RPE cells, very sensible because of high metabolic demand, needed for processes like physiological phagocytosis and life-long light illumination. Impairment of such functions could lead to pathological alterations like outer blood-retinal

barrier (BRB) dysfunctions, impairment of extracellular matrix (ECM) components, inhibition of photoreceptors outer segments processing, increasing of RPE cells senescence and/or apoptosis [43]. Moreover, several snoRNAs were already detected in murine retina, suggesting a possible involvement in retinal development and activity [14]. Although how such functions could be explicated need to be yet totally understood, several evidences highlight snoRNAs role in influencing translational fidelity, stop codon recognition and ribosome – ligand interactions [44]. Additionally, snoRNAs could play a fundamental role in chromatin remodeling, in miRNA – like post – transcriptional gene silencing and in lncRNA – like RNA splicing modulation [9]. Intriguingly, the most interesting snoRNAs research area regards the ability for pseudouridine in nonsense codon suppression, potentially altering the coding potential of non-canonical RNA substrates, such as mRNAs [45]. For this purpose, we realized a pathway analysis of snoRNAs host genes to highlight possible involvement in IRD onset and progression mechanisms. Host gene pathway analysis evidenced that editing of found snoRNAs might impair retinal neurotransmission. The link between synaptic communication and IRD etiopathogenesis has already been hypothesized [46], especially in relationship to ion channels structural and functional impairments [47]. Furthermore, recent studies highlighted the influence of oxidative stress in neurosignalling of the retinal cytotypes.

Conclusions

We performed a bulk RNA–Seq experiment on two group of RPE cells, treated with A2E and untreated, respectively, comparing snoRNA editing event changes in two selected time points (3h and 6h) over time zero. We found that 9 snoRNAs exhibited editing event variations in treated samples, targeting ribosomal RNA and with host genes involved in several biochemical pathways, all related to retinal neurotransmission. Nonetheless, many other important aspects have to be investigated. Predicted snoRNAs targets resulted from in silico analyses and, even if they are based on statistically significant algorithms and literature data, they will be further experimentally validated. Moreover, a deeper transcriptome sequencing on a larger number of samples could permit us to increase the number of detected snoRNAs, improving the knowledge on regulative functions of these small RNAs and IRDs.

References

1. Christofi, T. and A. Zaravinos. (2019). RNA editing in the forefront of epitranscriptomics and human health. J Transl Med, 17(1), 319.
2. Ramaswami, G. and J.B. Li. (2016). Identification of human RNA editing sites: A historical perspective. Methods, 107, 42-7.
3. Daniel, C., J. Lagergren, and M. Ohman. (2015). RNA editing of non-coding RNA and its role in gene regulation. Biochimie, 117, 22-7.
4. Gagnidze, K., V. Rayon-Estrada, S. Harroch, K. Bulloch, and F.N. Papavasiliou. (2018). A New Chapter in Genetic Medicine: RNA Editing and its Role in Disease Pathogenesis. Trends Mol Med, 24(3), 294-303.
5. Costa Cruz, P.H. and Y. Kawahara. (2021). RNA Editing in Neurological and Neurodegenerative Disorders. Methods Mol Biol, 2181, 309-330.
6. Scimone, C., P. Bramanti, C. Alafaci, F. Granata, F. Piva, C. Rinaldi, L. Donato, F. Greco, A. Sidoti, and R. D'Angelo. (2017). Update on Novel CCM Gene Mutations in Patients with Cerebral Cavernous Malformations. J Mol Neurosci, 61(2), 189-198.
7. Santosh, B., A. Varshney, and P.K. Yadava. (2015). Non-coding RNAs: biological functions and applications. Cell Biochem Funct, 33(1), 14-22.
8. Arai, T., S. Ito, and T. Suzuki. (2013). [Biogenesis and function of posttranscriptional modification of ribosomal RNA]. Seikagaku, 85(10), 896-908.
9. McMahon, M., A. Contreras, and D. Ruggero. (2015). Small RNAs with big implications: new insights into H/ACA snoRNA function and their role in human disease. Wiley Interdiscip Rev RNA, 6(2), 173-89.
10. Scott, M.S., M. Ono, K. Yamada, A. Endo, G.J. Barton, and A.I. Lamond. (2012). Human box C/D snoRNA processing conservation across multiple cell types. Nucleic Acids Res, 40(8), 3676-88.
11. Watkins, N.J. and M.T. Bohnsack. (2012). The box C/D and H/ACA snoRNPs: key players in the modification, processing and the dynamic folding of ribosomal

RNA. Wiley Interdiscip Rev RNA, 3(3), 397-414.

12. Dupuis-Sandoval, F., M. Poirier, and M.S. Scott. (2015). The emerging landscape of small nucleolar RNAs in cell biology. Wiley Interdiscip Rev RNA, 6(4), 381-97.
13. Stepanov, G.A., J.A. Filippova, A.B. Komissarov, E.V. Kuligina, V.A. Richter, and D.V. Semenov. (2015). Regulatory role of small nucleolar RNAs in human diseases. Biomed Res Int, 2015, 206849.
14. Liu, C.H., Z. Wang, Y. Sun, J.P. SanGiovanni, and J. Chen. (2016). Retinal expression of small non-coding RNAs in a murine model of proliferative retinopathy. Sci Rep, 6, 33947.
15. Diroma, M.A., L. Ciaccia, G. Pesole, and E. Picardi. (2021). Bioinformatics Resources for RNA Editing. Methods Mol Biol, 2181, 177-191.
16. Tran, S.S., Q. Zhou, and X. Xiao. (2020). Statistical inference of differential RNA-editing sites from RNA-sequencing data by hierarchical modeling. Bioinformatics, 36(9), 2796-2804.
17. Donato, L., C. Scimone, S. Alibrandi, C. Rinaldi, A. Sidoti, and R. D'Angelo. (2020). Transcriptome Analyses of lncRNAs in A2E-Stressed Retinal Epithelial Cells Unveil Advanced Links between Metabolic Impairments Related to Oxidative Stress and Retinitis Pigmentosa. Antioxidants (Basel), 9(4).
18. Blasiak, J. (2020). Senescence in the pathogenesis of age-related macular degeneration. Cell Mol Life Sci, 77(5), 789-805.
19. Scimone, C., L. Donato, S. Alibrandi, M. Vadala, G. Giglia, A. Sidoti, and R. D'Angelo. (2021). N-retinylidene-N-retinylethanolamine adduct induces expression of chronic inflammation cytokines in retinal pigment epithelium cells. Exp Eye Res, 209, 108641.
20. Rinaldi, C., L. Donato, S. Alibrandi, C. Scimone, R. D'Angelo, and A. Sidoti. (2021). Oxidative Stress and the Neurovascular Unit. Life (Basel), 11(8).
21. Donato, L., R. D'Angelo, S. Alibrandi, C. Rinaldi, A. Sidoti, and C. Scimone. (2020). Effects of A2E-Induced Oxidative Stress on Retinal Epithelial Cells: New Insights on Differential Gene Response and Retinal Dystrophies. Antioxidants (Basel), 9(4).
22. Sparrow, J.R., J. Zhou, S. Ben-Shabat, H. Vollmer, Y. Itagaki, and K. Nakanishi. (2002). Involvement of oxidative mechanisms in blue-light-induced damage to A2E-laden RPE. Invest Ophthalmol Vis Sci, 43(4), 1222-7.
23. Pham, T.N.M., C.Y. Shin, S.H. Park, T.H. Lee, H.Y. Ryu, S.B. Kim, K. Auh, and K.W. Jeong. (2021). Solanum melongena L.

Extract Protects Retinal Pigment Epithelial Cells from Blue Light-Induced Phototoxicity in In Vitro and In Vivo Models. Nutrients, 13(2).

24. Jin, H.L. and K.W. Jeong. (2022). Transcriptome Analysis of Long-Term Exposure to Blue Light in Retinal Pigment Epithelial Cells. Biomol Ther (Seoul), 30(3), 291-297.
25. Okonechnikov, K., A. Conesa, and F. Garcia-Alcalde. (2016). Qualimap 2: advanced multi-sample quality control for high-throughput sequencing data. Bioinformatics, 32(2), 292-4.
26. Ramaswami, G. and J.B. Li. (2014). RADAR: a rigorously annotated database of A-to-I RNA editing. Nucleic Acids Res, 42(Database issue), D109-13.
27. Lo Giudice, C., M.A. Tangaro, G. Pesole, and E. Picardi. (2020). Investigating RNA editing in deep transcriptome datasets with REDItools and REDIportal. Nat Protoc, 15(3), 1098-1131.
28. IBM. IBM SPSS Statistics for Windows, Version 26.0.
29. Bouchard-Bourelle, P., C. Desjardins-Henri, D. Mathurin-St-Pierre, G. Deschamps-Francoeur, E. Fafard-Couture, J.M. Garant, S.A. Elela, and M.S. Scott. (2020). snoDB: an interactive database of human snoRNA sequences, abundance and interactions. Nucleic Acids Res, 48(D1), D220-D225.
30. Bindea, G., B. Mlecnik, H. Hackl, P. Charoentong, M. Tosolini, A. Kirilovsky, W.H. Fridman, F. Pages, Z. Trajanoski, and J. Galon. (2009). ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics, 25(8), 1091-3.
31. Chatsirisupachai, K., D. Palmer, S. Ferreira, and J.P. de Magalhaes. (2019). A human tissue-specific transcriptomic analysis reveals a complex relationship between aging, cancer, and cellular senescence. Aging Cell, 18(6), e13041.
32. Roundtree, I.A., M.E. Evans, T. Pan, and C. He. (2017). Dynamic RNA Modifications in Gene Expression Regulation. Cell, 169(7), 1187-1200.
33. Chanfreau, G.F. (2017). Impact of RNA Modifications and RNA-Modifying Enzymes on Eukaryotic Ribonucleases. Enzymes, 41, 299-329.
34. Bianchetti, G., M.E. Clementi, B. Sampaolese, C. Serantoni, A. Abeltino, M. De Spirito, S. Sasson, and G. Maulucci. (2022). Investigation of DHA-Induced Regulation of Redox Homeostasis in Retinal Pigment Epithelium Cells through the Combination of Metabolic Imaging and Molecular Biology. Antioxidants (Basel), 11(6).

35. Clementi, M.E., G. Maulucci, G. Bianchetti, M. Pizzoferrato, B. Sampaolese, and G. Tringali. (2021). Cytoprotective Effects of Punicalagin on Hydrogen-Peroxide-Mediated Oxidative Stress and Mitochondrial Dysfunction in Retinal Pigment Epithelium Cells. Antioxidants (Basel), 10(2).
36. Adams, B.D., C. Parsons, L. Walker, W.C. Zhang, and F.J. Slack. (2017). Targeting noncoding RNAs in disease. J Clin Invest, 127(3), 761-771.
37. D'Angelo, R., C. Scimone, C. Rinaldi, G. Trimarchi, D. Italiano, P. Bramanti, A. Amato, and A. Sidoti. (2012). CCM2 gene polymorphisms in Italian sporadic patients with cerebral cavernous malformation: a case-control study. Int J Mol Med, 29(6), 1113-20.
38. D'Angelo, R., C. Alafaci, C. Scimone, A. Ruggeri, F.M. Salpietro, P. Bramanti, F. Tomasello, and A. Sidoti. (2013). Sporadic cerebral cavernous malformations: report of further mutations of CCM genes in 40 Italian patients. Biomed Res Int, 2013, 459253.
39. Scimone, C., P. Bramanti, A. Ruggeri, Z. Katsarou, L. Donato, A. Sidoti, and R. D'Angelo. (2015). Detection of Novel Mutation in Ccm3 Causes Familial Cerebral Cavernous Malformations. J Mol Neurosci, 57(3), 400-3.
40. Guzman-Aranguez, A., P. Loma, and J. Pintor. (2013). Small-interfering RNAs (siRNAs) as a promising tool for ocular therapy. Br J Pharmacol, 170(4), 730-47.
41. D'Angelo, R., L. Donato, I. Venza, C. Scimone, P. Aragona, and A. Sidoti. (2017). Possible protective role of the ABCA4 gene c.1268A>G missense variant in Stargardt disease and syndromic retinitis pigmentosa in a Sicilian family: Preliminary data. Int J Mol Med, 39(4), 1011-1020.
42. Campochiaro, P.A., R.W. Strauss, L. Lu, G. Hafiz, Y. Wolfson, S.M. Shah, R. Sophie, T.A. Mir, and H.P. Scholl. (2015). Is There Excess Oxidative Stress and Damage in Eyes of Patients with Retinitis Pigmentosa? Antioxid Redox Signal, 23(7), 643-8.
43. Mitter, S.K., C. Song, X. Qi, H. Mao, H. Rao, D. Akin, A. Lewin, M. Grant, W. Dunn, Jr., J. Ding, C. Bowes Rickman, and M. Boulton. (2014). Dysregulated autophagy in the RPE is associated with increased susceptibility to oxidative stress and AMD. Autophagy, 10(11), 1989-2005.
44. Wilusz, J.E. and J. Wilusz. (2014). Nonsense-mediated RNA decay: at the 'cutting edge' of regulated snoRNA production. Genes Dev, 28(22), 2447-9.
45. Weischenfeldt, J., J. Lykke-Andersen, and B. Porse. (2005). Messenger RNA

surveillance: neutralizing natural nonsense. Curr Biol, 15(14), R559-62.

46. Donato, L., S. Alibrandi, C. Scimone, C. Rinaldi, A. Dascola, A. Calamuneri, R. D'Angelo, and A. Sidoti. (2022). The impact of modifier genes on cone-rod dystrophy heterogeneity: An explorative familial pilot study and a hypothesis on neurotransmission impairment. PLoS One, 17(12), e0278857.

47. Donato, L., E.M. Abdalla, C. Scimone, S. Alibrandi, C. Rinaldi, K.M. Nabil, R. D'Angelo, and A. Sidoti. (2021). Impairments of Photoreceptor Outer Segments Renewal and Phototransduction Due to a Peripherin Rare Haplotype Variant: Insights from Molecular Modeling. Int J Mol Sci, 22(7).