Molecular Mechanisms Related to Oxidative Stress in Retinitis Pigmentosa

Carla Enrica Gallenga 1, Maria Lonardi 2, Sofia Pacetti 2, Sara Silvia Violanti 3, Paolo Tassinari 2, Francesco Di Virgilio 1,*, Mauro Tognon 1,† and Paolo Perri 4,*,†

Abstract: Retinitis pigmentosa (RP) is an inherited retinopathy. Nevertheless, non-genetic biological factors play a central role in its pathogenesis and progression, including inflammation, autophagy and oxidative stress. The retina is particularly affected by oxidative stress due to its high metabolic rate and oxygen consumption as well as photosensitizer molecules inside the photoreceptors being constantly subjected to light/oxidative stress, which induces accumulation of ROS in RPE, caused by damaged photoreceptor’s daily recycling. Oxidative DNA damage is a key regulator of microglial activation and photoreceptor degeneration in RP, as well as mutations in endogenous antioxidant pathways involved in DNA repair, oxidative stress protection and activation of antioxidant enzymes (MUTYH, CERKL and GLO1 genes, respectively). Moreover, exposure to oxidative stress alters the expression of micro-RNA (miRNAs) and of long non-codingRNA (lncRNAs), which might be implicated in RP etiopathogenesis and progression, modifying gene expression and cellular response to oxidative stress. The upregulation of the P2X7 receptor (P2X7R) also seems to be involved, causing pro-inflammatory cytokines and ROS release by macrophages and microglia, contributing to neuroinflammatory and neurodegenerative progression in RP. The multiple pathways analysed demonstrate that oxidative microglial activation may trigger the vicious cycle of non-resolved neuroinflammation and degeneration, suggesting that microglia may be a key therapy target of oxidative stress in RP.

Keywords: inflammation; retinitis pigmentosa; oxidative stress; P2X7R; micro-RNA; long non-coding RNA

1. Introduction

Retinitis pigmentosa (RP) is an inherited retinal degeneration caused by a collection of different genetic mutations, most of which are related to the progressive loss of photoreceptors (both rod and cone cells) and retinal pigmented epithelium (RPE) dysfunction [1]. This significant genetic burden is also associated with an important phenotypic heterogeneity, due to different penetrance, expressivity [2] and interaction with oxidative stress factors.

Cellular homeostasis requires a balance between oxidative species and antioxidant defence mechanisms. An excessive production of reactive oxygen species (ROS) and free radicals leads to oxidative stress, causing cellular dysfunction, necrosis, apoptosis or autophagic cell death [3,4].

The retina is particularly affected by oxidative stress due to different factors: high oxygen consumption related to a high metabolic demand, the presence of photoreceptors,
which contain photosensitizer molecules such as polyunsaturated fatty acids, and the accumulation of ROS in RPE, as an effect of damaged photoreceptor’s daily recycling [5].

Due to high metabolic activity, the retinal tissue has developed multiple defence mechanisms against oxidative damage. Oxidative stress (OS) is related to the activation of specific molecular pathways, such as PERK (PKR-like endoplasmic reticulum kinase) and IRE1 (inositol-requiring enzyme 1), that promote the transcription of genes encoding antioxidant enzymes. However, the chronic activation of some of these pathways causes mitochondrial damage in the long term, with intracellular accumulation of ROS resulting in retinal damage [6].

On the other side, inactivating mutations of genes involved in endogenous antioxidant defences leads to a rapid progression of the disease. In fact, in this review, we also analysed the role of GLO1 (Glyoxalase 1) and CERKL (ceramide-kinase like): mutations in these genes increase the sensitivity of retinal tissue to oxidative damage, resulting in cellular apoptosis and retinal neuro-degeneration due to the lack of resilience of the retinal tissue exposed to oxidative stress [7].

Among the defence mechanisms, the role of autophagy was also highlighted, prevalent in the retinal ganglion cells (RGC), which have a dual effect: reduction of intracellular ROS levels and mitochondrial support [8,9].

In a study conducted by Rodriguez-Muela et al. using a rd10 mouse model, it was shown that the calcium overload, the activation of calpain, the increase in cathepsin B activity, the reduced colocalization of cathepsin B with lysosomal markers and the reduction in the autophagosomal marker LC3-II (lipidated form of LC3-microtubule-associated protein 1A/1B-light chain 3) expression lead to an increase in permeability of the lysosomal membrane. All these changes in cellular activity, which occur before the death of photoreceptors, are markers of lysosomal dysfunction and down-regulation of autophagic activity [10].

Indeed, recent studies have discovered that the exposure to oxidative stress determines altered expression of micro-RNA (miRNAs) and of long non-coding RNA (lncRNAs) that might be implicated in the etiopathogenesis and progression of RP, since they alter gene expression along with the cellular response to oxidative stress [11].

This review focuses on the molecular mechanisms related to oxidative stress occurring in RP. These mechanisms play a central role in RP pathogenesis and progression.

Association between RP-Causative Mutations and Activation of UPR, PERK and IRE1 as a Response to Oxidative Stress

In response to environmental stress, such as OS, the translation of stress-inducible transcripts encoding heat shock proteins (HSP) is enhanced [3]. HSP perform chaperone functions by ensuring the correct folding of new proteins or refolding proteins that are damaged by OS.

A pathway that is responsible for cell translation reprogramming upon stress is PERK, a protein that is active in response to the accumulation of misfolded proteins [4].

Genetic mutations that were discovered to be causative of RP are involved in misfolding of transmembrane proteins implicated in photoreception and phototransduction. The accumulation of misfolded or unfolded proteins causes an increase in ROS, enhancing oxidative stress and the activation of an unfolded protein response (UPR), PERK and IRE1 pathways in photoreceptors cells. These pathways, involved in endogenous antioxidant defence, if chronically stimulated, determine the activation of pro-apoptotic programs associated with oxidative stress, pro-inflammatory signalling, dysfunctional autophagy, free cytosolic Ca\(^{2+}\) overload and an altered protein synthesis rate in the retina [4], leading to retinal degeneration [5] (Table 1).
2. Mutations in Endogenous Antioxidant Pathways: MUTYH, CERKL and GLO1

2.1. Role of 8-Oxoguanine and MUTYH in RP

One of the most prevalent genotoxic lesions is 8-oxoguanine (8-oxoG), and it is generated in DNA attacked by ROS [12]. MUTYH (mutY DNA glycosylase) plays an important role in the maintenance of genomic integrity through the activation of pathways involved in DNA repair. It removes adenine (A) from 8-oxoG:A mispairs, through the base excision repair (BER) pathway, preventing mutations in the genome [13].

MUTYH deficiency prevents single-strand breaks (SSBs) formation and cell death under oxidative stress [14,15]. However, under severe oxidative DNA damage, the excessive activation of MUTYH leads to formation of SSBs of DNA, causing disturbed homeostasis and cell death [16].

MUTYH-mediated BER is critical to promote retinal degeneration and inflammation in RP. As demonstrated by Oka et al. in a rd10 mice model, it occurs through two different pathways, (i) mitochondrial SSBs mediate calpain activation and (ii) nuclear SSBs induce poly (ADP-ribose) polymerase (PARP) activation [17].

The accumulation of 8-oxoG in the rd10 mouse retina is attenuated by hMTH1 overexpression and the activation of MUTYH, which leads to a reduction of rod and cone photoreceptor cell death [18], as well as microgliosis in rd10 mice [17]. Oxidized nucleic acids are increased in the photoreceptor layer but also in immune cells, such as microglial cells and macrophages, which infiltrate outer retinal regions. These findings suggest that oxidative DNA damage in the outer nuclear layer (ONL) of rd10 mice is derived from the oxidized nucleotide pool that occurs in photoreceptor cells and in other non-neuronal proliferating cells in the ONL, such as microglia, that may incorporate oxidized nucleic acids into their nuclear DNA during retinal degeneration, suggesting that these inflammatory cells could be an alternative source of ROS in RP [19].

In microglia, nuclear accumulation of 8-oxoG is associated with PARP activation occurring before the peak of photoreceptor degeneration; thereafter, it expands to the photoreceptor nuclei along with microglial activation [17]. Therefore, oxidative microglial activation may trigger the vicious cycle of non-resolved neuroinflammation and degeneration in RP as it happens in the brain [20], suggesting that the microglia, and especially the MUTYH-SSBs-PARP pathway, may be a key target of oxidative stress in RP (Table 1).

2.2. Oxidative Stress in RP: The Role of CERKL

CERKL (ceramide-kinase like) is a gene involved in the oxidative stress protection [21]. The precise function of CERKL is yet to be determined, but many studies show that it is implicated in the cellular response to oxidative stress and may play a role in protecting cells against stress injury [22,23]. The name of the gene stems from the diacylglycerol kinase domain, which shares homology with ceramide kinases.

Ceramide is a key sphingolipid (SL), a precursor of other bioactive and complex SLs lipid secondary messengers that control cell status [24,25] and plays a key role in stress-induced apoptosis [24].

To avoid entering apoptosis, induced by the increase of ceramide, cells activate enzymatic pathways involved in its clearance. In this context, the phosphorylation of ceramide, by ceramide kinase, produces a protective effect against apoptosis. Overexpression of CERKL isoforms protects cells from apoptosis induced by oxidative stress [21].

Mutations in CERKL, that have been reported to cause distinct RP [26,27], with characteristic macular and peripheral lesions and other cone-rod dystrophy (CRD), support the concept that failure in the endogenous mechanisms to overcome oxidative stress leads to an accelerated progression of retinal neurodegeneration (Table 1).

2.3. Glyoxalase 1 (GLO1) Related Genes and Pathways

Glyoxalase 1 (GLO1) is a ubiquitous cellular enzyme involved in detoxification of cytotoxic products of glycolysis, such as α-oxoaldehydes, methylglyoxal (MG), glyoxal (GO) and 3-deoxyglucosone (3-DG). In detail, GLO1 metabolizes MG and prevents MG-
induced damage. An excess of MG inactivates antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase (SOD) enzymes, impairing the degradation of MG, determining a positive feedback loop [28].

These cytotoxic products’ levels are increased in cells undergoing hyperglycemic metabolism, such as the RPE cells and photoreceptors, representing the principal source of intra- and extracellular advanced glycation end products (AGEs) [29]. High levels of AGEs, in addition to ROS, determine hyperinflammation and permanent tissue damage [30]. Intracellular AGE precursors, such as MG and GO, can also modify and inhibit the function of important enzymes, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and GLO1 [31].

AGEs exert their injuring effects by direct glycation of intracellular proteins and lipids, by the activation of cell signalling pathways through their binding to cellular receptors and the modulation of gene expression [32].

In the retina, AGEs could alter intra- and extracellular protein structure, increase inflammation and oxidative stress and, therefore, promote vascular dysfunction [33]. The retinal AGEs deposition could cause an upregulation of vascular endothelial growth factor (VEGF), a downregulation of pigment epithelium-derived factor (PEDF) and, eventually, a significant disruption of the inner blood–retinal barrier (iBRB) [34]. Additionally, increased advanced lipoxidation end-products (ALEs) accumulation was also detected in the outer retina. This portion contains photoreceptors, mainly rich in polyunsaturated fatty acids and therefore highly susceptible to lipid peroxidation [35].

In the retina, RPE cells exert the activity of protection by oxidative stress [36,37]. Numerous studies have validated the presence of high levels of ROS and AGEs in RPE, which are able to alter transduction pathways and gene expression [38].

Table 1. Altered pathway involved in endogenous antioxidant defence and their effects on retinal tissue.

| Pathway Involved                  | Effects                                                                 | Association with RP                                                                 | References            |
|----------------------------------|------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-----------------------|
| **CHRONIC ACTIVATION OF PERK AND IRE1** | Misfolded proteins: UPR activation                                     | Activation of pro-apoptotic programs Pro-inflammatory signalling Dysfunctional autophagy, free cytosolic Ca^{2+} | Altered protein synthesis rate in the retina and retinal degeneration [4,5] |
| **MUTYH MUTATION**               | DNA repair                                                             | Formation of single-strand breaks (SSBs) of DNA Disturbed homeostasis and cell death Oxidative microglial activation | Retinal degeneration and neuroinflammation in RP [16,18,20] |
| **CERKL MUTATION**               | Oxidative stress protection                                            | Activation of pro-apoptotic programs                                               | Accelerated progression of retinal neurodegeneration. [21,26,27] |
| **GLO1 MUTATION**                | Detoxification of cytotoxic products of glycolysis                     | Inactivation of antioxidant enzymes (glutathione peroxidase and SOD enzymes)        | Hyperinflammation and permanent tissue damage Vascular dysfunction Altered transduction pathways and genetic expression in EPR [28,30,33,38] |

GLO1 mutations, which are part of the endogenous detoxification system regulating ROS and AGE levels, contribute to the accumulation of AGEs in the retina, playing a role in RP pathogenesis [7,39] (Table 1).

Recent studies have identified 22 GLO1-related genes, with their related pathways, to be involved in a complex network of intracellular biochemical mechanisms that might be associated with RP onset and progression. Such pathways include microtubules and actin assembly, ubiquitin-proteasome activity, RE and Golgi integrity, vesicular trafficking, tran-
scriptional and translational control, glycolytic metabolism regulation and glycosylation modifications [40].

In fact, the global down-expression of these genes, excluding the upregulation of AUTS2 (cytoplasmatic activator of transcription and developmental regulator) and ANKH (progressive ankylosis protein homolog), could mostly lead to impairment in cell polarity and adhesion, through the alteration of actin filament structure and activity, with the final result being an increase in RPE apoptosis. Some of these genes are also involved in cell death through the dysregulation of energy metabolism or the translation machinery (SIK3, IPO3 and MRPS33).

The main mutations involving genes that compromise cellular metabolism, leading to RPE dysfunction and photoreceptor damage, which could accelerate retinal degeneration in RP, are explained below and summarized in Table 2.

2.3.1. AUTS2

Cytoplasmic activator of transcription and developmental regulator, AUTS2, is involved in the activation of the Rho family small GTPase Rac1. This pathway aims to control neuronal migration and neurite extension through the coordination of actin polymerization and microtubule dynamics. In the nucleus, AUTS2 functions as a transcriptional activator of many target genes, together with the polycomb complex 1 (PRC1) [41].

2.3.2. ARHGAP21 and PTPN13

Rho GTPase activating protein 21 (ARHGAP21) is involved in many pathways, both extra and intracellular, such as the inhibition of cell migration and proliferation, cell polarity, cell adhesion, Golgi regulation and positioning, intracellular trafficking and glucose homeostasis [42].

Protein tyrosine phosphatase non-receptor type 13 (PTPN13) is a tyrosine phosphatase which mediates phosphoinositide 3-kinase (PI3K) signalling through dephosphorylation of phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2) [43]. In addition, PTPN13 plays a relevant role in the same extracellular mechanism as ARHGAP21. Therefore, it controls negative apoptotic signalling [44] as the antagonist of Rho GTPase A, SLIT-ROBO Rho GTPase activating protein 1 (SRGAP1) [45].

2.3.3. FMNL2

Formin-like protein 2-FMNL2 is involved in the modulation of actin polymerization and organization of the cytoskeleton [46] through the regulation of contractility during epithelial junction maturation [47].

2.3.4. UBC, MYO18A, EPS15, ANKH

Ubiquitin C (UBC), Myosin XVIII A (MYO18A), epidermal growth factor receptor pathway substrate 15 (EPS15) and ANKH inorganic pyrophosphate transport regulator are also involved in intracellular transport processes, and the dysregulation of these genes could influence vesicular trafficking of RPE cells, essential for photoreceptor outer segment (POS) renewal, visual cycle intermediate regeneration and avoiding AGE accumulation.

Specifically, MYO18A is involved in actin’s retrograde treadmilling and its transport from focal adhesions to the leading edge [48]. ANKH regulates trans-Golgi network trafficking and endocytosis [49], and finally, EPS15, with its encoded protein, is involved in clathrin-coated pit maturation, including invagination or budding and cell growth regulation [50,51].

2.3.5. RFFL, FBXW2, CAND1

These three genes are involved in the ubiquitin-proteasome system activity, and their down-regulation could lead to ER stress, inducing the accumulation of misfolded proteins and AGEs.
**RFFL** (ring finger and FYVE-like domain containing E3 ubiquitin protein ligase /rififylin) is an E3 ubiquitin-protein ligase which plays an important role in the extrinsic pathway of apoptosis, modulating cellular death domain receptors [52,53].

**FBXW2** (F-box/WD repeat-containing protein 2) enhances the ubiquitylation and degradation of β-catenin, overexpressed in the WNT/β-catenin pathway during inflammatory processes.

**CAND1** (Cullin-associated NEDD8-dissociated protein 1) controls the tubules’ elongation and retraction, thereby regulating the tubular endoplasmic reticulum network [54].

The down-expression of **FBXW2** and **CAND1** also plays a role in ROS production and cell death, altering cellular respiration processes through the impairment of glycolytic metabolism in RPE cells.

### 2.3.6. SIK3

**SIK** family kinase 3 (**SIK3**) gene encodes for a specific kinase that up-regulates mTOR, CREB signalling and cholesterol biosynthesis, correlating with retinoid metabolism and melanogenesis [55].

The down-expression of **SIK3** could cause a defect in energy metabolism, decreasing mitochondrial respiration and up-regulating autophagy in order to remove dysfunctional cellular components, leading to cellular antioxidant mechanisms’ impairment [56,57].

### 2.3.7. IPO7

Importin 7 (**IPO7**) functions as a receptor for nuclear localization signals (NLS) and promotes translocation of import substrates through the nuclear pore complex (NPC). Its down-regulation could trigger p53-dependent growth arrest, ribosomal biogenesis stress and nucleolar morphology changes [58].

### 2.3.8. MRPS33, MORC4, MCPH1, NFIA, CTIF and LMBRD1

Down-expression of mitochondrial ribosomal protein S33 (**MRPS33**) could damage mitochondrial protein synthesis [59]. **MORC** family CW-type zinc finger 4 (**MORC4**) and Microcephalin 1 (**MCPH1**) dysregulation arrests DNA damage repair and causes apoptosis [60,61]. Reduced levels of nuclear Factor I A (**NFIA**) impair mitotic exit and cell differentiation [62]. Cap-binding complex-dependent translation initiation factor (**CTIF**) down-expression alters the intermodulation between translation and the aggresome-autophagy pathway, compromising mRNA and protein’s quality control [63]. Lastly, **LMBR1** domain containing 1 (**LMBRD1**) down-regulation could affect the transport and metabolism of cobalamin, decreasing distribution of cyanocobalamin (vitamin B12) from the blood to the retina [64,65].

**Table 2. GLO1-related genes mutations: RPE dysfunction and photoreceptors damage.**

| Gene                      | Effects of Mutation                                      | Consequences               | References                  |
|---------------------------|----------------------------------------------------------|----------------------------|-----------------------------|
| **AUTS2, ANKH**           | Alteration of actin filament structure and activity      | RPE apoptosis              | [41]                        |
| **SIK3, IPO3, MRPS33**    | Dysregulation of energy metabolism and translation machinery | Cell death                 | [56,58,59]                  |
| **ARHGAP2, PTPN13**       | Alteration of cell migration and proliferation           | RPE apoptosis              | [42–45]                     |
|                           | Modification of cell polarity, cell adhesion,            |                             |                             |
|                           | Golgi regulation                                         |                             |                             |
|                           | Impairment of intracellular trafficking and glucose homeostasis |                             |                             |
| **FMNL2**                 | Alteration of actin polymerization and organization of the cytoskeleton | Alteration of vesicular trafficking of RPE cells | [46,47]                    |
Table 2. Cont.

| Gene                  | Effects of Mutation                              | Consequences                                                                 | References |
|-----------------------|-------------------------------------------------|-------------------------------------------------------------------------------|------------|
| UBC, MYO18A, EPS15, ANKH | Impairment of intracellular transport processes | Influence of vesicular trafficking of RPE cells (essential for POS renewal and visual cycle intermediate regeneration) AGE accumulation | [48–51]    |
| RFFL, FBXW2, CAND1    | ER stress                                       | Accumulation of misfolded proteins AGEs and ROS production                     | [52–54]    |
| SIK3                  | Defect in energy metabolism                     | Decrease of mitochondrial respiration Up-regulation of autophagy              | [56,57]    |
| IPO7                  | Ribosomal biogenesis stress                     | Nucleolar morphology changes p53-dependent growth arrest                      | [58]       |
| MRPS33, MORC4, MCPH1, NFIA, CTIF, LMBRD1 | Damage of mitochondrial protein synthesis Arrests in DNA damage repair Impairment of mitotic exit and cell differentiation Impairment of mRNA and protein quality control Alteration of transport and metabolism of cobalamin | RPE apoptosis and retinal degeneration Decreased distribution of cyanocobalamin (vitamin B12) from blood to retina | [59,62–64] |

3. miRNA Altered Expression and Oxidative Stress:

miRNAs represent a group of short non-coding RNAs that are involved in transcript degradation or translational inhibition of their target mRNAs, whose function is to regulate post-transcriptional gene expression [66]. They are key regulators of many important biological processes [67], controlling specific pathways by targeting networks of functionally correlated genes.

Alterations of miRNA expression, due to mutations in either the miRNA itself or its target genes, could lead to several pathological conditions.

There is much evidence to support the role of miRNAs in normal retinal development and functions [68]: deletion of specific retina-enriched miRNAs has relevant effects on the development of retinal diseases, such as RP [69].

Luigi Donato et al. investigated the complexity of human retina miRNome (murine miRNA transcriptome), analysing data from human RPE cell transcriptomes.

Due to its specific proteins, RPE has many functions, including (i) the regeneration of outer segments of photoreceptors by phagocytizing the spent discs, (ii) regulating the trafficking of nutrients and waste products to and from the retina, (iii) protecting the outer retina from excessive high-energy light and the subsequent light-generated reactive oxygen species and (iv) maintaining retinal homeostasis thanks to the release of diffusible factors.

As a result of all this metabolic activity, RPE cells are very susceptible to oxidative stress [36,70]. Furthermore, RPE cells contain a significant number of mitochondria that are the principal cause of ROS production and removal inside the cell [70]. OS plays a critical role in the etiopathogenesis of RP [71] and leads to pathobiological changes in RPE cells [72] determining outer blood–retina barrier dysfunction [73], inhibition of processing of photoreceptor outer segments by RPE [74], expression of transforming growth factor-β2 [75] and synthesis alterations of extracellular matrix components [76]. All these changes lead to increased RPE apoptosis [77] and senescence changes [72,78].
In a recent paper [11], authors compared changes in the expression of miRNAs obtained from whole transcriptome analyses between two groups of RPE cells, one untreated and the other exposed to the oxidant agent oxidized low-density lipoprotein (oxLDL). In the treated samples, 23 miRNAs revealed altered expression, targeting genes involved in several biochemical pathways, many of which were associated with RP. Moreover, five RP causative genes (KLHL7, RDH11, CERKL, AIPL1 and USH1G) emerged as already confirmed targets of five altered miRNAs (hsa-miR-1307, hsa-miR-3064, hsa-miR-4709, hsa-miR-3615 and hsa-miR-637), suggesting a connection between induced oxidative stress and RP development and progression.

The finding of new regulative functions of miRNAs, and especially their altered expression induced by OS in RPE, should lead to the discovery of alternative mechanism responsible of the etiopathogenesis and progression of RP.

4. Role of Long Non-Coding RNA

Long non-coding RNAs (lncRNAs) are untranslated transcripts that regulate many biological processes through epigenetic modifications, RNA splicing, mRNA decay and mRNA translation [79], acting as scaffolds for chromatin-modifying complexes [80].

Recent studies have highlighted the close connection between OS, the biochemical pathway involved in RP pathogenesis, and lncRNAs differential expression [81,82] in RPE metabolism.

In cells such as RPE, the high metabolic demand determines the up-regulation of DNA metabolic processes that cause an increased rDNA silencing, due to chromatin-associated lncRNAs, that leads to cellular growth alterations and RPE cell death [83].

In this process, lncRNAs have a key role, as it is well-known that they are involved in DNA metabolic processes: lncRNAs up- or down-regulation could alter gene expression, along with cellular responses to OS, which is one of the most important pathways involved in RP.

Considering DNA damage, two different lncRNAs are likely induced and overexpressed: MNX-AS1 and MIR31HG.

They interact with Cyclin D1 mRNA, whose encoding gene is already known to transcribe a specific lncRNA involved in DNA damage condition, acting as transcription repressors [84].

Oxidative stress also determines changes in glucose metabolism. Specifically, the dysregulation of two lnc-RNAs that are implicated in bioenergetic reactions related to glucose, BDNF-AS and TUG1 [85], were discovered to induce RPE apoptosis [86,87].

Moreover, high glucose levels influence the synthesis of IGF-1, PEDF, AGEs and their receptors (RAGE) that determine OS and inflammatory reactions, leading to retinal degeneration [88].

Additionally, other deregulated lncRNAs connected with insulin-related pathways, such as ARF, AKT1 [89], CRNDE, CYTOR CAP1 and ACACA [90,91], were discovered to induce RPE cell apoptosis [37].

Considering lipid metabolism and homeostasis, RPE cells present intracellular signalling pathways whose gene transcription is regulated by the peroxisome proliferator-activated receptor (PPAR) [92].

In the study by Donato et al. [37], three lncRNAs (AC007283.1, AC012442.2 and AC089983) were identified as being involved in fatty acids biosynthesis and metabolism. In particular, the down-expression of AC007283.1 and AC012442.2, along with the over-expression AC089983.1, could alter the gene expression and lipid metabolism regulation by PPAR-alfa.

Additionally, several down-regulated lncRNAs, such as AC004943.2 and AC007036.3, were found to interact with various miRNAs involved in fatty acid metabolism and biosynthesis, leading to the impairment of the integrity and functionality of lipidic retinal structures [37].
Lastly, the involvement of numerous lncRNAs was also identified in protein metabolism. It is well-known that protein’s misfolding, including those related to retinal survival and vision process, like rhodopsin, determine the disruptions of cellular protein homeostasis [93] and could lead to cell death.

Two clusters made of dysregulated lncRNAs and their interactors/host genes were detected to be involved in cellular amide metabolism [37]. Among them, the up-regulation of PTEN-induced putative kinase protein 1 (PINK1) antisense RNA and the downregulation of FMRP translational regulator 1 (FMR1)-IT1 sense intronic and vimentin (VIM) antisense 1 RNA were found to be particularly interesting.

PINK1 regulates mitochondrial damage, promotes mitophagy and protects cells from death and apoptosis, especially during high glucose-mediated regulation of RPE [91], reflecting the apoptosis status of RPE.

FMR1-IT1 and VIM-AS1 are related to synaptogenesis, intracellular trafficking and cellular stability [94,95], representing the attempt of RPE cells to boost the production of vital proteins.

5. P2X7 Receptor and Inflammation in RP

The P2X7 receptor (P2X7R) is an ATP-gated ion channel expressed by immune and inflammatory cells and is over expressed during inflammation and by stressed or dying cells involved in innate and adaptive immune responses. It is recognized as a potent trigger of ROS production, and its over-stimulation leads to the impairment of mitochondrial metabolism, caspase activation as well as apoptosis induction [96,97]. Its stimulation also induces ATP release by means of a membrane pore formation or in association with pannexin hemichannels, activating the NLRP3 (NLR family pyrin domain-containing 3) inflammasome that induces the maturation and release of pro-inflammatory cytokines (IL-1β and IL-18) and the production of ROS, released by macrophages and microglia, contributing to the progression of neuroinflammatory and neurodegenerative diseases [98,99].

The expression of P2X7R was showed in several components of the retinal layers: not only on photoreceptor cells, but also on retinal ganglion cell, amacrine and horizontal cells, microglia and Müller glial cells, astrocytes and pericytes as well as RPE cells [100].

An upregulation of P2x7R mRNA was demonstrated within the retina of an RP mouse model when photoreceptor degeneration occurred. Furthermore, intravitreal administration of ATP in this animal model caused photoreceptor cell death and loss of function due to the activation of P2x7R. As a demonstration, this process can be delayed by intravitreal injection of a P2x7 receptor antagonist in rd1 mouse models [101].

6. The Dual Role of Microglia in RP: Between Neurotoxicity and Neuroprotection

We have already analysed two mechanisms that determine an activation of microglia, involving MUTYH and P2X7 receptors. The excessive activation of MUTYH determines nuclear accumulation of 8-oxoG, which is the most prevalent genotoxic lesion, and the PARP pathway as a consequence of accumulation of oxidized nucleic acids, while the upregulation of P2X7R is induced by stressed retinal cells that release extracellular ATP, leading to microglial chemotaxis and activation [100].

Herein, we report in detail the molecular mechanism underlying the activation of microglia and the role of these cells in the development of RP.

The microglia is composed of immune cells implicated in neuronal homeostasis and innate immune defences. Microglia’s cells are activated in response to the altered physiology of mutation-bearing photoreceptors [102], inducing the production of proinflammatory cytokines and chemokines [103–105].

A study conducted on a rd1 mouse line demonstrates that in the early stages of disease, there is a persistent upregulation of microglial markers, such as Tmem119, C1qa, TNF, Ili1a and Ili1b, that act as inflammatory factors, determining neurotoxicity [106–109]. A rd1 mouse line treated with PLX5622, a potent colony-stimulator factor 1 receptor (CSF1R) inhibitor that eliminates microglial population, did not show an increase of these factors.
However, it is known that the role of microglia in the development of RP is dictated by a balance between neurotoxic and neuroprotective/neurotrophic influences.

In fact, the activation of retinal microglia induces the expression of neurotrophic factors in Muller cells, exerting neuroprotection in degenerating photoreceptors [110]. Furthermore, it has been demonstrated that overexpression of TGF-beta, an anti-inflammatory cytokine that has a modulatory effect on the action of microglia [111], exercises a protective effect on the degeneration of the cones.

More specifically, through RNA-seq, it has been highlighted that the effects are explicit through post-translational modifications of the proteome not detectable using RNA-seq.

Inhibition of this neuroprotective pathway, induced by the expression of TGF-beta, determines microglial activation, resulting in degenerative changes in retinal tissue given by the expression of proinflammatory cytokines [112].

Finally, the last mechanism concerns the interaction between microglia and complement activation, specifically between the central complement component (C3) and the microglia-expressed receptor (CR3) [113].

An increased C3 expression was found in microglia translocated in ONL after rod degeneration, with a concomitant opsonization of the degenerating photoreceptor by iC3b, a product of C3 activation. This complement activation is therefore a microglial response aimed at phagocytosis of the apoptotic photoreceptor and the restoration of homeostasis. When both C3 and CR3 are deficient, it causes an increased proinflammatory cytokine expression, accumulation of apoptotic cells and neurodegeneration [114].

These findings could lead to other possible immunomodulatory therapy opportunities.

7. Conclusions

The molecular mechanisms related to oxidative stress occurring in retinitis pigmentosa (Figure 1) play a central role in its pathogenesis and progression. The burden of the analysed pathways demonstrate that oxidative microglial activation may trigger the vicious cycle of non-resolved neuroinflammation and degeneration in RP, suggesting that the microglia may be a key target of oxidative stress in RP. Failure of the endogenous mechanisms to overcome oxidative stress leads to an accelerated progression of retinal neurodegeneration.

Figure 1. Main molecular mechanisms occurring during oxidative stress in RP.
This analytic review aimed to highlight possible therapeutic targets inside the different pathogenetic mechanisms that induce the formation of ROS and potentially slow down the evolution of the disease over time.

**Funding:** The works of the authors cited herein have been funded by AIRC grants to FDV and MT, and University of Ferrara, FAR projects to FDV, MT and PP.

**Conflicts of Interest:** The authors declare no conflict of interest.

**Abbreviations**

| Abbreviation | Definition |
|--------------|------------|
| 3-DG | 3-DeoxyGlucosone |
| 8-oxoG | 8oxoGuanine |
| ACACA | Acetyl-CoA Carboxylase Alpha |
| AGEs | Advanced Glycation End products |
| AIP1 | Aryl Hydrocarbon Receptor Interacting Protein Like 1 |
| AKT1 | AKT Serine/Threonine Kinase 1 |
| ALKs | Advanced lipoxidation End-products |
| ANKH | Progressive ankylosis protein homolog |
| ARF | Alternate Reading Frame |
| ARHGAP21 | Rho GTPase Activating Protein 21 |
| AUTS2 | Cytoplasmatic activator of transcription and developmental regulator |
| BDNF-AS | Brain-derived neurotrophic factor Antisense |
| BER | Base Excision Repair |
| C3 | Complement Component 3 |
| CAND1 | Cullin-associated NEDD8-dissociated protein 1 |
| CAP1 | Cyclase Associated Actin Cytoskeleton Regulatory Protein 1 |
| CERKL | Ceramide Kinase Like |
| CR3 | Complement Receptor 3 |
| CRD | Cone-Rode Dystrophy |
| CREB | cAMP response element-binding protein |
| CRNDE | Colorectal Neoplasia Differentially Expressed |
| CSF1R | Colony Stimulator Factor 1 Receptor (PLX5622) |
| CTIF | Cap Binding Complex Dependent Translation Initiation Factor |
| CYTOR | Cytoskeleton Regulator RNA |
| EP515 | Epidermal Growth Factor Receptor Pathway Substrate 15 |
| FBXW2 | F-box/WD repeat-containing protein 2 |
| FMNL2 | Formin Like Protein 2 |
| FMR1 | FMRP Translational Regulator 1 |
| GAPDH | Glyceraldehyde 3-phosphate Dehydrogenase |
| GLO1 | Glyoxalase 1 |
| GO | Glyoxal |
| hMTH1 | Human MutT Homologue |
| HSP | Heat Shock Protein |
| iBRB | Inner Blood-Retinal Barrier |
| IGF1 | insulin-like growth factor-1 |
| IL-18 | interleukin-18 |
| IL-1β | interleukin-1β |
| IPO3 | Transportin-3 |
| IPO7 | Importin 7 |
| IRE1 | Inositol-Requiring Enzyme 1 |
| KLHL7 | Kelch Like Family Member 7 |
| LC3-II | Lipidated form of LC3 (Microtubule-associated protein 1A/1B-light chain 3) |
| LMBRD1 | Limb Development Membrane Protein Domain 1 |
| lnc-RNA | long non-coding-RNA |
| MCPH | Microcephalin |
| MG | MethylGlyoxal |
| MIR31HG | MicroRNA 31 Host Gene |
miRNA microRNA
miRNome Murine miRNA
MNX Motor Neuron And Pancreas Homeobox 1
MNX-AS1 antisense transcript of MNX1
MORC 4 MORC family CW-type zinc finger protein 4
MRPS33 28S ribosomal protein S33
mTOR Mammalian target of Rapamycin
MUTYH mutY DNA glycosylase
MYO18A Myosin XVIII A
NFIA Nuclear Factor I A
NLS Nuclear Localization Signals
NPC Nuclear Pore Complex
ONL Outer Nuclear Layer
OS Oxidative Stress
P2X7R P2X 7 receptor
PARP Poly(ADP-ribose) polymerase
PEDF Pigment Epithelium-derived Factor
PERK PKR-like Endoplasmic Reticulum Kinase
PI3K Phosphoinositide 3 Kinase
PINK1 PTEN-induced kinase 1
POS Photoreceptor Outer Segment
PPAR peroxisome proliferator-activated receptor
PRC1 Polycomb Complex 1
PTEN Phosphatase and tensin homolog
PTPN13 Protein Tyrosine Phosphatase Non-Receptor type 13
RAC1 Rac Family Small GTPase 1
RDH11 Retinol Dehydrogenase 11
RE Rough Endoplasmic reticulum
RFFL E3 ubiquitin-protein ligase riffylin
ROS Reactive Oxygen Species
RPE Retinal Pigment Epithelium
SIK3 SIK Family Kinase 3
SL SphingoLipid
SOD Superoxide Dismutase
SRGAP1 Slit-Robo Rho GTPase Activating Protein 1
SSB Single Strand Breaks
TUG1 Taurine Up-Regulated 1
UBC Ubiquitin C
UPR Unfolded Protein Response
USH1G Usher syndrome type-1G protein
VEGF Vascular Endothelial Growth Factor
VIM Vimentin

References
1. Campa, C.; Gallenga, C.E.; Bolletta, E.; Perri, P. The Role of Gene Therapy in the Treatment of Retinal Diseases: A Review. Curr. Gene Ther. 2017, 17, 194–213. [CrossRef] [PubMed]
2. Sorrentino, F.S.; Gallenga, C.E.; Bonifazzi, C.; Perri, P. A challenge to the striking genotypic heterogeneity of retinitis pigmentosa: A better understanding of the pathophysiology using the newest genetic strategies. Eye 2016, 30, 1542–1548. [CrossRef] [PubMed]
3. Sies, H.; Jones, D.P. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat. Rev. Mol. Cell Biol. 2020, 21, 363–383. [CrossRef] [PubMed]
4. Gorbatyuk, M.S.; Starra, C.R.; Gorbatyuk, O.S. Endoplasmic reticulum stress: New insights into the pathogenesis and treatment of retinal degenerative diseases. Prog. Retin. Eye Res. 2020, 79, 100860. [CrossRef]
5. Doménech, E.B.; Marfany, G. The Relevance of Oxidative Stress in the Pathogenesis and Therapy of Retinal Dystrophies. Antioxidants 2020, 9, 347. [CrossRef]
6. Kroeger, H.; Chiang, W.C.; Felden, J.; Nguyen, A.; Lin, J.H. ER stress and unfolded protein response in ocular health and disease. FEBs J. 2019, 286, 399–412. [CrossRef]
7. Donato, L.; Scimone, C.; Nicocia, G.; Denaro, L.; Robledo, R.; Sidoti, A.; D’Angelo, R. GLO1 gene polymorphisms and their association with retinitis pigmentosa: A case–control study in a Sicilian population. Mol. Biol. Rep. 2018, 45, 1349–1355. [CrossRef]
8. Boya, P. Why autophagy is good for retinal ganglion cells? *Eye* 2017, 31, 185–190. [CrossRef]

9. 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. [CrossRef] [PubMed]

10. Rodríguez-Muela, N.; Hernández-Pinto, A.M.; Serrano-Puebla, A.; García-Ledo, L.; Latorre, S.H.; de la Rosa, E.J.; Boya, P. Lyssosomal membrane permeabilization and autophagy blockade contribute to photoreceptor cell death in a mouse model of retinitis pigmentosa. *Cell Death Differ.* 2015, 22, 476–487. [CrossRef]

11. Donato, L.; Bramanti, P.; Scimone, C.; Rinaldi, C.; Giorgianni, F.; Beranova-Giorgianni, S.; Koirała, D.; D’Angelo, R.; Sidoti, A. miRNAexpression profile of retinal pigment epithelial cells under oxidative stress conditions. *FEBS Open Bio* 2018, 8, 219–233. [CrossRef]

12. Jingwen, C.; Zhenqian, H.; Xin, W.; Jaqi, K.; Yan, R.; Wei, G.; Xiang, L.; Jingmei, W.; Weidong, D.; Yusaku, N.; et al. Oxidative stress induces different tissue dependent effects on Mutyh-deficient mice. *Free Radic. Biol. Med.* 2019, 143, 482–493. [PubMed]

13. Douglas, M.; Nunez, N.N.; Burnside, M.A.; Bradshaw, K.M.; David, S.S. Repair of 8-oxoG: A Mismatches by the MUTYH Glycosylase: Mechanism, Metals and medicine. *Free Radic. Biol. Med.* 2017, 107, 202–215. [PubMed]

14. Foti, J.J.; Devadoss, B.; Winkler, J.A.; Collins, J.J.; Walker, G.C. Oxidation of the guanine nucleotide pool underlies cell death by bactericidal antibiotics. *Science* 2012, 336, 315–319. [CrossRef] [PubMed]

15. Nakatake, S.; Murakami, Y.; Ikeda, Y.; Morioka, N.; Tachibana, T.; Fujiiwara, K.; Yoshida, N.; Notomi, S.; Hisatomi, T.; Yoshida, S.; et al. MUTOH promotes oxidative microglial activation and inherited retinal degeneration. *JCI Insight* 2016, 1, e87781. [CrossRef]

16. Oka, S.; Nakabeppu, Y. DNA glycosylase encoded by MUTYH functions as a molecular switch for programmeed cell death under oxidative stress to suppress tumorigenesis. *Cancer Sci.* 2011, 102, 677–682. [CrossRef] [PubMed]

17. Oka, S.; Ohno, M.; Tsuchimoto, D.; Sakumi, K.; Furuichi, M.; Nakabeppu, Y. Two distinct pathways of cell death triggered by oxidative damage to nuclear and mitochondrial DNAs. *EMBO J.* 2008, 27, 421–432. [CrossRef]

18. Murakami, Y.; Ikeda, Y.; Yoshida, N.; Notomi, S.; Hisatomi, T.; Oka, S.; De Luca, G.; Yonemitsu, Y.; Bignami, M.; Nakabeppu, Y.; et al. MutT homolog-1 attenuates oxidative DNA damage and delays photoreceptor cell death in inherited retinal degeneration. *Am. J. Pathol.* 2012, 181, 1378–1386. [CrossRef] [PubMed]

19. Usui, S.; Oveson, B.C.; Lee, S.Y.; Jo, Y.J.; Yoshida, T.; Miki, A.; Miki, K.; Iwase, T.; Lu, L.; Campochiaro, P.A. NADPH oxidase plays a central role in cone cell death in retinitis pigmentosa. *J. Neurochem.* 2009, 109, 1028–1037. [CrossRef]

20. Conti, P.; Lauritano, D.; Caraffa, A.; Gallenga, C.E.; Kritas, S.K.; Ronconi, G.; Martinotti, S. Microglia and mast cells generate proinflammatory cytokines in the brain and worsen inflammatory state: Suppressor effect of IL-37. *Eur. J. Pharmacol.* 2020, 15, 875. [CrossRef]

21. Tuson, M.; Garanto, A.; González-Duarte, R.; Marfany, G. Overexpression of CERKL, a gene responsible for retinitis pigmentosa in humans, protects cells from apoptosis induced by oxidative stress. *Mol. Vis.* 2009, 15, 168–180. [CrossRef]

22. Fatinajafabadi, A.; Pérez-Jiménez, E.; Riera, M.; Knecht, E.; González-Duarte, R. CERKL, a retinal disease gene, encodes an mRNA-binding protein that localizes in compact and untranslated mRNPs associated with microtubules. *PLoS ONE* 2014, 9, e87886. [CrossRef] [PubMed]

23. Li, C.; Wang, L.; Zhang, J.; Huang, M.; Wong, F.; Liu, X.; Liu, F.; Cui, X.; Yang, G.; Chen, J.; et al. CERKL interacts with mitochondrial TRX2 and protects retinal cells from oxidative stress-induced apoptosis. *Biochim. Biophys. Acta Mol. Basis Dis.* 2014, 1842, 1121–1129. [CrossRef]

24. Hannun, Y.A.; Obeid, L.M. The ceramide-centric universe of lipid-mediated cell regulation: Stress encounters of the lipid kind. *J. Biol. Chem.* 2002, 277, 25847–25850. [CrossRef]

25. Hannun, Y.A.; Obeid, L.M. Principles of bioactive lipid signalling: Lessons from sphingolipids. *Nat. Rev. Mol. Cell Biol.* 2008, 9, 139–150. [CrossRef] [PubMed]

26. Tuson, M.; Marfany, G.; González-Duarte, R. Mutation of CERKL, a novel human ceramide kinase gene, causes autosomal recessive retinitis pigmentosa (RP26). *Am. J. Hum. Genet.* 2004, 74, 128–138. [CrossRef] [PubMed]

27. Auslender, N.; Sharon, D.; Abbasi, A.H.; Garzoozi, H.J.; Banin, E.; Ben-Yosef, T. A common founder mutation of CERKL underlies autosomal recessive retinal degeneration with early macular involvement among Yemenite Jews. *Investig. Ophthalmol. Vis. Sci.* 2007, 48, 5431–5438. [CrossRef]

28. Khan, M.A.; Anwar, S.; Alijabor, A.N.; Al-Orainy, M.; Aldebas, Y.H.; Islam, S.; Younus, H. Protective effect of thymoquinone on glucose or methylglyoxal-induced glycation of superoxide dismutase. *Int. J. Biol. Macromol.* 2014, 65, 16–20. [CrossRef]

29. Groener, J.B.; Okonomou, D.; Choko, R.; Kender, Z.; Zemva, J.; Kühn, L.; Muckenthaler, M.; Peters, V.; Fleming, T.; Kopf, S.; et al. Methylglyoxal and Advanced Glycation End Products in Patients with Diabetes—What We Know so Far and the Missing Links. *Exp. Clin. Endocrinol. Diabetes* 2019, 127, 497–504. [CrossRef] [PubMed]

30. Nedic, O.; Rattan, S.I.; Grune, T.; Trougakos, I.P. Molecular effects of advanced glycation end products on cell signalling pathways, ageing and pathophysiology. *Free Radic. Res.* 2013, 47, 28–38. [CrossRef]

31. Lee, H.J.; Howell, S.K.; Sanford, J.R.; Beisswenger, P.J. Methylglyoxal can modify GAPDH activity and structure. *Ann. N. Y. Acad. Sci.* 2005, 1043, 135–145. [CrossRef]

32. Wautier, M.P.; Guillausseau, P.J.; Wautier, J.L. Activation of the receptor for advanced glycation end products and consequences on health. *Diabetes Metab. Syndr.* 2017, 11, 305–309. [CrossRef]

33. Xu, J.; Chen, L.J.; Yu, J.; Wang, H.J.; Zhang, F.; Liu, Q.; Wu, J. Involvement of Advanced Glycation End Products in the Pathogenesis of Diabetic Retinopathy. *Cell Physiol. Biochem.* 2018, 48, 705–717. [CrossRef]
34. Grigsby, J.G.; Allen, D.M.; Ferrigno, A.S.; Vellanki, S.; Pouw, C.E.; Hejny, W.A.; Tsin, A.T.C. Autocrine and Paracrine Secretion of Vascular Endothelial Growth Factor in the Pre-Hypoxic Diabetic Retina. *Curr. Diabetes Res.* 2017, 13, 161–174. [CrossRef]
35. Chen, M.; Curtis, T.M.; Stitt, A.W. Advanced glycation end products and diabetic retinopathy. *Curr. Med. Chem.* 2013, 20, 3234–3240. [CrossRef]
36. Datta, S.; Cano, M.; Ebrahimi, K.; Wang, L.; Handa, J.T. The impact of oxidative stress and inflammation on RPE degeneration in non-vascular AMD. *Prog. Retin. Eye Res.* 2017, 60, 201–218. [CrossRef] [PubMed]
37. Donato, L.; Scimone, C.; Alibrandi, S.; Rinaldi, C.; Sidoti, A.; D’Angelo, R. Transcriptome Analyses of lncRNAs in A2E-Stressed Retinal Epithelial Cells Unveil Advanced Links between Metabolic Impairments Related to Oxidative Stress and Retinitis Pigmentosa. *Antioxidants* 2020, 9, 318. [CrossRef] [PubMed]
38. Kaarmiranta, K.; Koskela, A.; Felszeghy, S.; Kivinen, N.; Salmiminen, A.; Kauppinen, A. Fatty acids and oxidized lipoproteins contribute to autophagy and innate immunity responses upon the degeneration of retinal pigment epithelial and development of age-related macular degeneration. *Biochimie* 2019, 159, 49–54. [CrossRef] [PubMed]
39. Peculis, R.; Konrade, I.; Skapare, E.; Fridmanis, D.; Nikitina-Zake, L.; Lejnieks, A.; Pirags, V.; Dambrova, M.; Klovins, J. Identification of glyoxalase 1 polymorphisms associated with enzyme activity. *Gene* 2013, 515, 140–143. [CrossRef] [PubMed]
40. Donato, L.; Scimone, C.; Alibrandi, S.; Nicocia, G.; Rinaldi, C.; Sidoti, A.; D’Angelo, R. Discovery of GLO1 New Related Genes and Pathways by RNA-Seq on A2E-Stressed Retinal Epithelial Cells Could Improve Knowledge on Retinitis Pigmentosa. *Antioxidants* 2020, 9, 416. [CrossRef] [PubMed]
41. Horii, K.; Hoshino, M. Neuronal Migration and AUTS2 Syndrome. *Brain Sci.* 2017, 7, 54. [CrossRef] [PubMed]
42. Rosa, L.R.O.; Soares, G.M.; Silveira, L.R.; Boscheroto, A.C.; Barbosa-Sampaio, H.C.L.; ARHGAP21 as a master regulator of multiple cellular processes. *J. Cell Physiol.* 2018, 233, 8477–8481. [CrossRef] [PubMed]
43. Kuchay, S.; Duan, S.; Schenkein, E.; Pescharioli, A.; Saraf, A.; Florens, L.; Washburn, M.P.; Pagano, M. FBXL2- and PTPL1-mediated degradation of p110-free p85beta regulatory subunit controls the PI(3)K signalling cascade. *Nat. Cell Biol.* 2013, 15, 472–480. [CrossRef] [PubMed]
44. Villa, F.; Deak, M.; Bloomber, G.B.; Alessi, D.R.; van Aalten, D.M. Crystal structure of the PTPL1/FAP-1 human tyrosine phosphatase mutated in colorectal cancer: Evidence for a second phosphotyrosine substrate recognition pocket. *J. Biol. Chem.* 2005, 280, 8180–8187. [CrossRef] [PubMed]
45. Kaur, H.; Xu, N.; Doycheva, D.M.; Malaguit, J.; Tang, J.; Zhang, J.H. Recombinant Slit2 attenuates neuronal apoptosis via the Robo1-srGAP1 pathway in a rat model of neonatal HIE. *Neuropsychopharmacology* 2019, 158, 107727. [CrossRef]
46. Kage, F.; Steffen, A.; Ellinger, A.; Ranftler, C.; Gehre, C.; Brakebusch, C.; Pavelka, M.; Stradal, T.; Rottner, K. FNML2 and -3 regulate Golgi architecture and anterograde transport downstream of Cdc42. *Sci. Rep.* 2017, 7, 9791. [CrossRef] [PubMed]
47. Liang, X.; Kiru, S.; Gomez, G.A.; Yap, A.S. Regulated recruitment of SRGAP1 modulates RhoA signaling for contractility during epithelial junction maturation. *Cytoskeleton* 2018, 75, 61–69. [CrossRef]
48. Buschman, M.D.; Field, S.J. MYO18A: An unusual myosin. *Adv. Biol. Regul.* 2018, 46, 87–92. [CrossRef] [PubMed]
49. Van Gils, M.; Nollet, L.; Verly, E.; Deianova, N.; Vanakker, O.M. Cellular signaling in pseudoxanthoma elasticum: An update. *Exp. Cell Res.* 2004, 312, 933–942. [CrossRef] [PubMed]
50. Majumdar, A.; Ramagiri, S.; Rikhy, R. Drosophilina homologue of Eps15 is essential for synaptic vesicle recycling. *Exp. Cell Res.* 2006, 312, 2288–2298. [CrossRef]
51. Gan, X.; Wang, C.; Patel, M.; Kreutz, B.; Zhou, M.; Kozasa, T.; Wu, D. Different Raf protein kinases mediate different signaling pathways to stimulate E3 ligase RFFL gene expression in cell migration regulation. *J. Biol. Chem.* 2013, 288, 33978–33984. [CrossRef] [PubMed]
52. Sakai, R.; Fukuda, R.; Unida, S.; Aki, M.; Ono, Y.; Endo, A.; Kusumi, S.; Koga, D.; Fukushima, T.; Komada, M.; et al. The integral function of the endocytic recycling compartment is regulated by RFFL-mediated ubiquitylation of Rab11 effectors. *J. Cell Sci.* 2019, 132. [CrossRef]
53. Kajiho, H.; Yamamoto, Y.; Sakasaka, T. CAND1 regulates lunapark for the proper tubular network of the endoplasmic reticulum. *Sci. Rep.* 2019, 9, 13152. [CrossRef] [PubMed]
54. Uebi, T.; Itoh, Y.; Hatano, O.; Kumagai, A.; Sanosaka, M.; Sasaki, T.; Sasagawa, S.; Doi, J.; Tatsumi, K.; Mitamura, K.; et al. Involvement of SIK3 in glucose and lipid homeostasis in mice. *PLoS ONE* 2019, 14, e0219198. [CrossRef] [PubMed]
55. Kriete, A.; Bosl, W.J.; Booker, G. Rule-based cell systems model of aging using feedback loop motifs mediated by stress responses. *PLoS Comput. Biol.* 2010, 6, e1000820. [CrossRef] [PubMed]
56. Chong, C.M.; Zheng, W. Artemisinin protects human retinal pigment epithelial cells from hydrogen peroxide-induced oxidative damage through activation of ERK/CREB signaling. *Redox Biol.* 2016, 9, 50–56. [CrossRef] [PubMed]
57. Liang, X.; Kiru, S.; Gomez, G.A.; Yap, A.S. Regulated recruitment of SRGAP1 modulates RhoA signaling for contractility during epithelial junction maturation. *Cytoskeleton* 2018, 75, 61–69. [CrossRef]
58. Alibrandi, S.; Rinaldi, C.; Sidoti, A.; D’Angelo, R. Discovery of GLO1 New Related Genes and Pathways by RNA-Seq on A2E-Stressed Retinal Epithelial Cells Could Improve Knowledge on Retinitis Pigmentosa. *Antioxidants* 2020, 9, 318. [CrossRef] [PubMed]
59. Donato, L.; Scimone, C.; Alibrandi, S.; Nicocia, G.; Rinaldi, C.; Sidoti, A.; D’Angelo, R. Transcriptome Analyses of lncRNAs in A2E-Stressed Retinal Epithelial Cells Unveil Advanced Links between Metabolic Impairments Related to Oxidative Stress and Retinitis Pigmentosa. *Antioxidants* 2020, 9, 416. [CrossRef] [PubMed]
60. Horii, K.; Hoshino, M. Neuronal Migration and AUTS2 Syndrome. *Brain Sci.* 2017, 7, 54. [CrossRef] [PubMed]
61. Rosa, L.R.O.; Soares, G.M.; Silveira, L.R.; Boscheroto, A.C.; Barbosa-Sampaio, H.C.L.; ARHGAP21 as a master regulator of multiple cellular processes. *J. Cell Physiol.* 2018, 233, 8477–8481. [CrossRef] [PubMed]
62. Kuchay, S.; Duan, S.; Schenkein, E.; Pescharioli, A.; Saraf, A.; Florens, L.; Washburn, M.P.; Pagano, M. FBXL2- and PTPL1-mediated degradation of p110-free p85beta regulatory subunit controls the PI(3)K signalling cascade. *Nat. Cell Biol.* 2013, 15, 472–480. [CrossRef] [PubMed]
63. Liang, X.; Kiru, S.; Gomez, G.A.; Yap, A.S. Regulated recruitment of SRGAP1 modulates RhoA signaling for contractility during epithelial junction maturation. *Cytoskeleton* 2018, 75, 61–69. [CrossRef]
64. Buschman, M.D.; Field, S.J. MYO18A: An unusual myosin. *Adv. Biol. Regul.* 2018, 46, 87–92. [CrossRef] [PubMed]
65. Van Gils, M.; Nollet, L.; Verly, E.; Deianova, N.; Vanakker, O.M. Cellular signaling in pseudoxanthoma elasticum: An update. *Cell Signal.* 2019, 55, 119–129. [CrossRef]
66. Khanobdee, K.; Kolberg, J.B.; Dunlevy, J.R. Nuclear and plasma membrane localization of SH3BP4 in retinal pigment epithelial cells. *Mol. Vis.* 2004, 10, 933–942. [CrossRef]
60. Yang, Z.; Zhuang, Q.; Hu, G.; Geng, S. MORC4 is a novel breast cancer oncogene regulated by miR-193b-3p. J. Cell Biochem. 2019, 120, 4634–4643. [CrossRef] [PubMed]

61. Liu, X.; Zong, W.; Li, T.; Wang, Y.; Xu, X.; Zhou, Z.W.; Wang, Z.Q. The E3 ubiquitin ligase APC/C(Cdh1) degrades MCPH1 after MCPH1-betaTrCP2-Cdc25A-mediated mitotic entry to ensure neurogenesis. EMBO J. 2017, 36, 3666–3681. [CrossRef] [PubMed]

62. Clark, B.S.; Stein-O’Brien, G.L.; Shiu, F.; Cannon, G.H.; Davis-Marcisak, E.; Sherman, T.; Santiago, C.P.; Hoang, T.V.; Rajaii, F.; James-Exposito, R.E.; et al. Single-Cell RNA-Seq Analysis of Retinal Development Identifies NFI Factors as Regulating Mitotic Exit and Late-Born Cell Specification. Neuron 2019, 102, 1111–1126. [CrossRef] [PubMed]

63. Park, Y.; Park, J.; Kim, Y.K. Crosstalk between translation and the aggresome-autophagy pathway. Autophagy 2018, 14, 1079–1081. [CrossRef]

64. Kawaguchi, K.; Okamoto, T.; Morita, M.; Imanaka, T. Translocation of the ABC transporter ABCD4 from the endoplasmic reticulum to lysosomes requires the escort protein LMBDI. Sci. Rep. 2016, 6, 30183. [CrossRef] [PubMed]

65. Kinoshita, Y.; Nogami, K.; Jomura, R.; Akanuma, S.I.; Abe, H.; Inouye, M.; Kubo, Y.; Hosoya, K.I. Investigation of Receptor-Mediated Cyanocobalamin (Vitamin B12) Transport across the Inner Blood-Retinal Barrier Using Fluorescence-Labeled Cyanocobalamin. Mol. Pharm. 2018, 15, 3583–3594. [CrossRef]

66. He, L.; Hannon, G.J. MicroRNAs: Small RNAs with a big role in gene regulation. Nat. Rev. Genet. 2004, 5, 522–531. [CrossRef]

67. Zamore, P.D. Ribonome: The big world of small RNAs. [CrossRef]

68. Ohana, R.; Weiman-Kelman, B.; Raviv, S.; Tamm, E.R.; Pasmanik-Chor, M.; Rinon, A.; Netanely, D.; Shamir, R.; Solomon, A.S. The E3 ubiquitin ligase APC/C(Cdh1) degrades MCPH1 after MCPH1-betaTrCP2-Cdc25A-mediated mitotic entry to ensure neurogenesis. [CrossRef]

69. Park, Y.; Park, J.; Kim, Y.K. Crosstalk between translation and the aggresome-autophagy pathway. Autophagy 2018, 14, 1079–1081. [CrossRef]

70. Tian, B.; Maidana, D.E.; Dib, B.; Miller, J.B.; Bouzika, P.; Miller, J.W.; Vavvas, D.G.; Lin, H. MiR-17-3p Exacerbates Oxidative Damage in Human Retinal Pigment Epithelial Cells. PLoS ONE 2016, 11, e0160887. [CrossRef] [PubMed]

71. Kruk, J.; Kubasik-Kladna, K.; Aboul-Enein, H.Y. The role oxidative stress in the pathogenesis of eye diseases: Current status and a dual role of physical activity. Mini Rev. Med. Chem. 2015, 16, 241–257. [CrossRef] [PubMed]

72. Mao, K.; Shu, W.; Qiu, Q.; Gu, Q.; Wu, X. Salvianolic acid A protects retinal pigment epithelium from OX-LDL-induced inflammation in an age-related macular degeneration model. Discov. Med. 2017, 23, 129–147. [PubMed]

73. Kim, J.H.; Lee, S.J.; Kim, K.W.; Yu, Y.S.; Kim, J.H. Oxidized low density lipoprotein-induced senescence of retinal pigment epithelial cells is followed by outer blood-retinal barrier dysfunction. Int. J. Biochem. Cell Biol. 2012, 44, 808–814. [CrossRef] [PubMed]

74. Hoppe, G.; Marmorstein, A.D.; Pennock, E.A.; Hoff, H.F. Oxidized low density lipoprotein-induced inhibition of processing of photoreceptor outer segments by RPE. Invest. Ophthalmol. Vis. Sci. 2001, 42, 2714–2720.

75. Yu, A.L.; Lorenz, R.L.; Haritoglou, C.; Kampik, A.; Welge-Lussen, U. Biological effects of native and oxidized low-density lipoproteins in cultured human retinal pigment epithelial cells. Exp. Eye Res. 2009, 88, 495–503. [CrossRef] [PubMed]

76. Saneipour, M.; Ghatreh-Samani, K.; Heydarian, E.; Farrokh, E.; Abdian, N. Adiponectin inhibits oxidized low density lipoproteins in cultured human retinal pigment epithelial cells. Avitabile, T.; et al. Retinal and circulating miRNAs in age-related macular degeneration: An in vivo animal and human study. Front. Pharmacol. 2017, 8, 168. [CrossRef] [PubMed]

77. Tian, B.; Maidana, D.E.; Dib, B.; Miller, J.B.; Bouzika, P.; Miller, J.W.; Vavvas, D.G.; Lin, H. MiR-17-3p Exacerbates Oxidative Damage in Human Retinal Pigment Epithelial Cells. PLoS ONE 2016, 11, e0160887. [CrossRef] [PubMed]

78. Bailey, T.A.; Kanuga, N.; Romero, I.A.; Greenwood, J.; Luthert, P.J.; Cheetham, M.E. Oxidative stress affects the junctional integrity of retinal pigment epithelial cells. Invest. Ophthalmol. Vis. Sci. 2004, 45, 675–684. [CrossRef] [PubMed]

79. Kopp, F.; Mendell, J.T. Functional Classification and Experimental Dissection of Long Noncoding RNAs. [CrossRef]

80. Kondo, Y.; Shinjo, K.; Katsushima, K. Long non-coding RNAs as an epigenetic regulator in human cancers. Cancer Sci. 2017, 108, 1927–1933. [CrossRef]

81. Wawrzyniak, O.; Zarebska, Z.; Rolke, K.; Gotz-Wieckowska, A. Circular and long non-coding RNAs and their role in ophthalmologic diseases. Acta Biochim. Pol. 2018, 65, 497–508. [CrossRef] [PubMed]

82. Donato, L.; Scimone, C.; Rinaldi, C.; D’Angelo, R.; Sidoti, A. Non-coding RNAome of RPE cells under oxidative stress suggests unknown regulative aspects of Retinitis pigmentosa etiopathogenesis. Sci. Rep. 2018, 8, 16638. [CrossRef]

83. Manelyte, L.; Strohner, R.; Gross, T.; Langst, G. Chromatin targeting signals, nucleosome positioning mechanism and non-coding RNA-mediated regulation of the chromatin remodeling complex NsdC. PLoS Genet. 2014, 10, e1004157. [CrossRef]

84. Thapar, R.; Regulation of DNA Double-Strand Break Repair by Non-Coding RNAs. Molecules 2018, 23, 2789. [CrossRef] [PubMed]

85. Han, X.; Yang, Y.; Sun, Y.; Qin, L.; Yang, Y. LncRNA TUG1 affects cell viability by regulating glycolysis in osteosarcoma cells. Gene 2018, 674, 87–92. [CrossRef] [PubMed]

86. Chen, S.; Wang, M.; Yang, H.; Mao, L.; He, Q.; Jin, H.; Ye, Z.M.; Luo, X.Y.; Xia, Y.P.; Hu, B. LncRNA TUG1 sponges microRNA-9 to promote neurons apoptosis by up-regulated Bcl211 under ischemia. Biochem. Biophys. Res. Commun. 2017, 485, 167–173. [CrossRef]

87. Li, Y.; Xu, F.; Xiao, H.; Han, F. Long noncoding RNA BDNF-AS inversely regulated BDNF and modulated high-glucose induced apoptosis in human retinal pigment epithelial cells. J. Cell Biochem. 2018, 119, 817–823. [CrossRef] [PubMed]
Antioxidants 2021, 10, 848

88. Kang, M.K.; Lee, E.J.; Kim, Y.H.; Kim, D.Y.; Oh, H.; Kim, S.I.; Kang, Y.H. Chrysin Ameliorates Malfunction of Retinoid Visual Cycle through Blocking Activation of AGE-RAGE-ER Stress in Glucose-Stimulated Retinal Pigment Epithelial Cells and Diabetic Eyes. *Nutrients* **2018**, *10*, 1046. [CrossRef] [PubMed]

89. Millar, C.A.; Powell, K.A.; Hickson, G.R.; Bader, M.F.; Gould, G.W. Evidence for a role for ADP-riboseylation factor 6 in insulin-stimulated glucose transporter-4 (GLUT4) trafficking in 3T3-L1 adipocytes. *J. Biol. Chem.* **1999**, *274*, 17619–17625. [CrossRef] [PubMed]

90. Ellis, B.C.; Graham, L.D.; Molloy, P.L. CRNDE, a long non-coding RNA responsive to insulin/IGF signaling, regulates genes involved in central metabolism. *Biochim. Biophys. Acta* **2014**, *1843*, 372–386. [CrossRef]

91. Zhang, Y.; Xi, X.; Mei, Y.; Zhao, X.; Zhou, L.; Ma, M.; Liu, S.; Zha, X.; Yang, Y. High-glucose induces retinal pigment epithelium mitochondrial pathways of apoptosis and inhibits mitophagy by regulating ROS/PINK1/Parkin signal pathway. *Biomed. Pharmacother.* **2019**, *111*, 1315–1325. [CrossRef] [PubMed]

92. Yanagi, Y. Role of Peroxisome Proliferator Activator Receptor gamma on Blood Retinal Barrier Breakdown. *PPAR Res.* **2008**, *2008*, doi. [CrossRef] [PubMed]

93. Lin, J.H.; Lavail, M.M. Misfolded proteins and retinal dystrophies. *Adv. Exp. Med. Biol.* **2017**, *894*, 115–121. [PubMed]

94. Lundkvist, A.; Reichenbach, A.; Betsholtz, C.; Carmeliet, P.; Wolburg, H.; Pekny, M. Under stress, the absence of intermediate filaments from Muller cells in the retina has structural and functional consequences. *J. Cell Sci.* **2004**, *117*, 3481–3488. [CrossRef] [PubMed]

95. Rossignol, R.; Ranchon-Cole, I.; Paris, A.; Herzine, A.; Perche, A.; Laurenceau, D.; Bertrand, P.; Cercy, C.; Pichon, J.; Mortaud, S.; et al. Visual sensorial impairments in neurodevelopmental disorders: Evidence for a retinal phenotype in Fragile X Syndrome. *PLoS ONE* **2014**, *9*, e105996. [CrossRef]

96. Adinolfi, E.; Giuliani, A.L.; De Marchi, E.; Pegoraro, A.; Orioli, E.; Di Virgilio, F. The P2X7 receptor: A main player in inflammation. *Biochem. Pharmacol.* **2018**, *151*, 234–244. [CrossRef] [PubMed]

97. Di Virgilio, F.; Tang, Y.; Sarti, A.C.; Rossato, M. A rationale for targeting the P2X7 receptor in Coronavirus disease 19. *Br. J. Pharmacol.* **2020**, *177*, 4990–4994. [CrossRef] [PubMed]

98. Di Virgilio, F.; Dal Ben, D.; Sarti, A.C.; Giuliani, A.L.; Falzoni, S. The P2X7 Receptor in Infection and Inflammation. *Immunity* **2017**, *47*, 15–31. [CrossRef]

99. Savio, L.E.B.; De Andrade Mello, P.; Da Silva, C.G.; Coutinho-Silva, R. The P2X7 Receptor in Inflammatory Diseases: Angel or Demon? *Front. Pharmacol.* **2018**, *9*, 52. [PubMed]

100. Reichenbach, A.; Bringmann, A. Purinergic signaling in retinal degeneration and regeneration. *Neuropharmacology* **2016**, *104*, 194–211. [CrossRef] [PubMed]

101. Calzaferri, F.; Ruiz-Ruiz, C.; Diego, A.M.G.; Pascual, R.; Ménendez-López, I.; Cano-Abad, M.F.; García, A.G. The purinergic P2X7 receptor as a potential drug target to combat neuroinflammation in neurodegenerative diseases. *Med. Res. Rev.* **2020**, *40*, 2427–2465. [CrossRef] [PubMed]

102. Appelbaum, T.; Santana, E.; Aguirre, G.D. Strong Upregulation of Inflammatory Genes Accompanies Photoreceptor demise in Canine Models of Retinal Degeneration. *PLoS ONE* **2017**, *12*, e0177224.

103. Block, M.L.; Zecca, L.; Hong, J.S. Microglia-mediated neurotoxicity: Uncovering the molecular mechanism. *Nat. Rev. Neurosci.* **2007**, *8*, 57–69. [CrossRef] [PubMed]

104. Subbramaniam, C.S.; Wang, C.; Hu, Q.; Dheen, S.T. Microglia-mediated neuroinflammation in neurodegenerative diseases. *Semin. Cell Dev. Biol.* **2019**, *94*, 112–120. [CrossRef] [PubMed]

105. Peng, B.; Xiao, J.; Wang, K.; So, K.F.; Tipeo, G.L.; Lin, B. Suppression of microglial activation is neuroprotective in a mouse model of human retinitis pigmentosa. *J. Neurosci.* **2014**, *34*, 8139–8150. [CrossRef] [PubMed]

106. Wang, S.K.; Xue, Y.; Čepko, C.L. Microglia modulation by TGF-Beta1 protects cones in mouse models of retinal degeneration. *J. Clin. Invest.* **2020**, *130*, 4360–4369. [PubMed]

107. Smith, J.A.; Da, A.; Ray, S.K.; Banik, N.L. Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. *Brain Res. Bull.* **2012**, *87*, 10–20. [CrossRef]

108. Liddelow, S.A.; Guntenplan, K.A.; Clarke, L.E.; Bennett, F.C.; Bohlen, C.J.; Schirmer, L.; Bennett, M.L.; Münch, A.E.; Chung, W-S.; Peterson, T.C.; et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* **2017**, *541*, 481–487. [CrossRef]

109. Chitnis, T.; Weiner, H.L. CNS inflammation and neurodegeneration. *J. Clin. Invest.* **2017**, *127*, 3577–3587. [CrossRef]

110. Taylor, R.A.; Chang, C.F.; Goods, B.A.; Hammond, M.D.; Mac Groy, B.; Ai, Y.; Steinschneider, A.F.; Renfroe, S.C.; Askenase, M.H.; McCullough, L.D.; et al. TGF-beta1 modulates microglial phenotype and promotes recovery after intracerebral hemorrhage. *J. Clin. Invest.* **2017**, *127*, 280–292. [CrossRef] [PubMed]

111. O’Koren, E.G.; Yu, C.; Klingeborn, M.; Wong, A.Y.; Prigge, C.L.; Mathew, R.; Kalnitsky, J.; Msallam, R.A.; Silvin, A.; Kay, J.N.; et al. Microglial function is distinct in different anatomical locations during retinal homeostasis and degeneration. *Immunity* **2019**, *50*, 723–737. [CrossRef] [PubMed]
113. Rutar, M.; Valter, K.; Natoli, R.; Provis, J.M. Synthesis and propagation of complement C3 by microglia/monocytes in the aging retina. PLoS ONE. 2014, 9, e93343. [CrossRef] [PubMed]

114. Silverman, S.M.; Ma, W.; Wang, X.; Zhao, L.; Wong, W.T. C3- and CR3- dependent microglial clearance protects photoreceptors in retinitis pigmentosa. J. Exp. Med. 2019, 216, 1925–1943. [CrossRef]