

Review

Metabolic pathways in context: mTOR signalling in the retina and optic nerve

Anthony Yao MBBS¹ & Peter van Wijngaarden PhD FRANZCO^{1,2}

1. Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, 32 Gisborne St, East Melbourne, Victoria, Australia
2. Ophthalmology, Department of Surgery, University of Melbourne, Melbourne, Australia

Correspondence: Peter van Wijngaarden, Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, Melbourne, Australia

Email: peterv@unimelb.edu.au

Short running title: Retinal and optic nerve mTOR signalling

Received 22 April 2020; accepted 5 July 2020

Funding sources / Financial disclosure: The Centre for Eye Research Australia receives Operational Infrastructure Support from the Victorian Government.

Conflict of interest: None

Ethic statement: None required.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1111/ceo.13819](https://doi.org/10.1111/ceo.13819)

ABSTRACT

The mechanistic target of rapamycin (mTOR) signalling network plays a key role in growth and development, autophagy, metabolism, inflammation as well as ageing, and it is therefore important in ocular health and disease. mTOR dysregulation has been identified in a range of conditions, including age-related macular degeneration, diabetic retinopathy, retinitis pigmentosa, traumatic optic neuropathy and glaucoma. Experimental modulation of the pathway has contributed to the understanding of these diseases and offers the potential for new avenues of therapy. This review discusses the mTOR pathway and its role in health and in diseases of the retina and optic nerve.

Keywords: Metabolism, mTOR, Retina, Optic nerve

1. BACKGROUND

1.1. Dirty origins

The mechanistic target of rapamycin (mTOR) is a highly conserved serine/threonine protein kinase that plays a central role in cellular growth, metabolism and autophagy. Accordingly, the mTOR pathway has wide-ranging implications for systemic as well as ocular health and disease. Understanding of the pathway blossomed following the discovery of a potent inhibitor compound isolated from a soil bacterium. The *Streptomyces* genus of bacteria has been an important tool for drug discovery for decades, being the source of a wide variety of antimicrobial, immunosuppressant, anti-cancer and antihypertensive compounds¹. Analysis of a sample of *Streptomyces hygroscopicus*, isolated from the soil of Easter Island (Rapa Nui) in the 1970s, identified a macrolide antibiotic named rapamycin in recognition of its site of origin. The compound was initially considered to be significant for its antifungal properties, however over the subsequent years the immunosuppressive properties of rapamycin gained attention and the drug was alternately named sirolimus, after the discovery of its structural similarities with the immunosuppressant tacrolimus². Further research revealed that rapamycin acts by binding to immunophilin FK-506-binding protein 12 (FKBP12) and inhibits phosphorylation of a signalling complex named the mechanistic target of rapamycin (mTOR) complex 1, after the drug³.

This review provides an introduction to the signalling pathway and its importance in eye health and in diseases. It does not intend to provide an exhaustive account of the intricacies of the pathway and its regulation. Readers interested in a more detailed interrogation of mTOR signalling are directed to a number of authoritative reviews that have been published on the subject^{4,5,6}.

1.2. An introduction to the mTOR pathway

In mammals, mTOR forms the core component of two structurally and functionally distinct signalling complexes. mTOR complex 1 (mTORC1) is the metabolic “master regulator”, involved in both anabolism and catabolism as a mediator of protein synthesis, cell growth and autophagy. It consists of three core proteins including mTOR and regulatory protein associated with mTOR (Raptor), as well as protein regulators such as the proline-rich AKT substrate (PRAS40)^{5,7}. In contrast, mTOR complex 2 (mTORC2) is less sensitive to rapamycin, and promotes cell survival via phosphorylation of the AGC protein kinase family (PKA/PKG/PKC) and regulates cytoskeleton dynamics⁷, as well as playing a role in neovascularisation⁸. It consists of three core components including mTOR and rapamycin-insensitive companion of mTOR (rictor)^{4,7}.

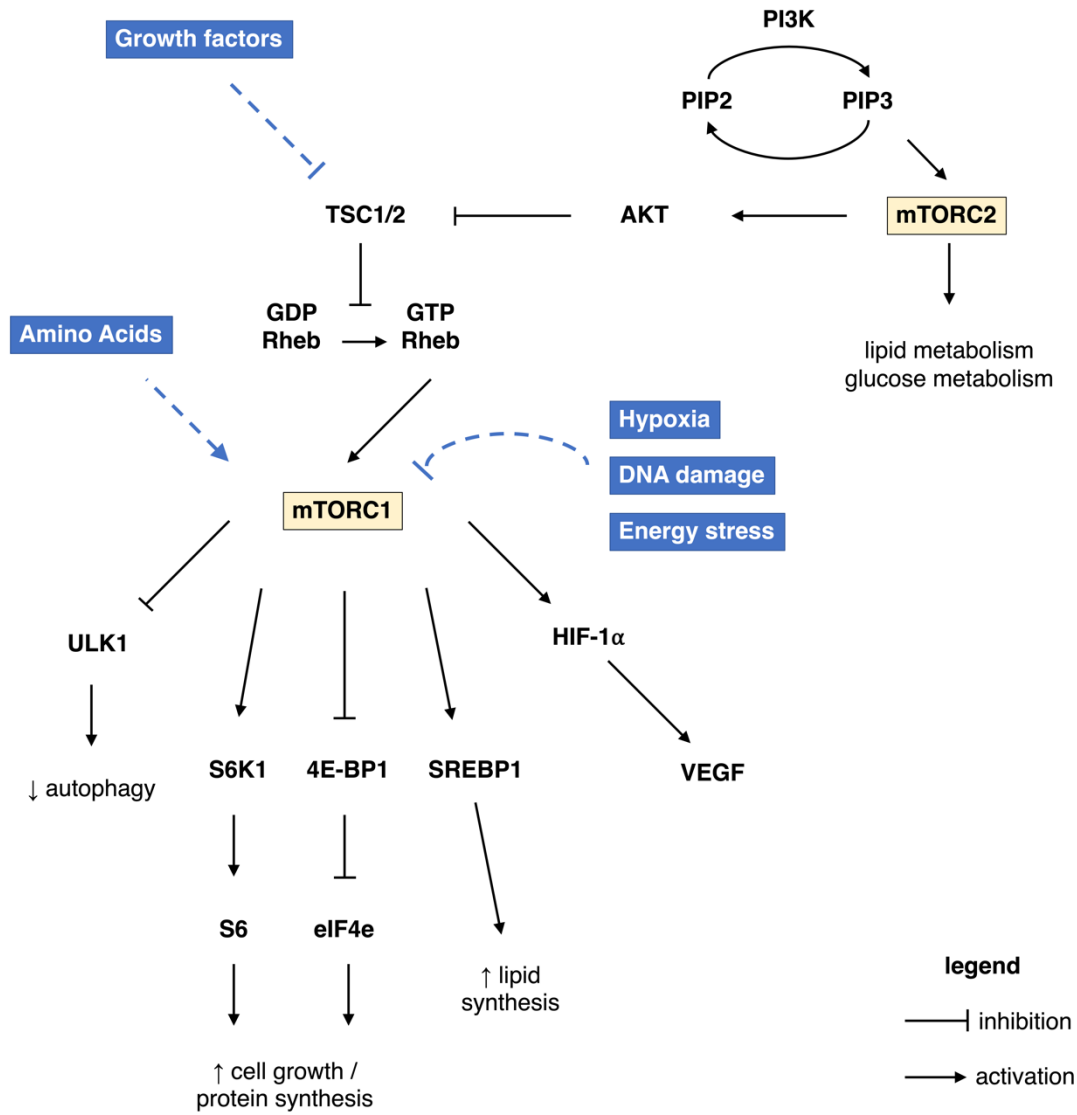
1.3. Upstream regulators

The mTOR complexes play central roles within cells in the integration of a diverse range of signals. The mTORC1 signalling network senses inputs from growth factors and cytokines, as well as levels of energy substrates, amino acids and oxygen⁵. In the context of adequate substrate and energy conditions, mTORC1 signalling mediates a wide range of anabolic processes. The tuberous sclerosis complex (TSC) is a key negative regulator of mTORC1. It inactivates the Ras homolog enriched in brain (Rheb) GTP-ase, suppressing mTORC1 activity⁶. The insulin/insulin-like growth factor 1 (IGF1) pathway activates mTORC1 via the phosphorylation and inactivation of TSC at multiple sites – through protein kinase B (AKT/PKB), extracellular-signal-regulated kinase 1/2 (ERK1/2) and ribosomal S6 kinase (RSK1). By phosphorylating and dissociating the inhibitory PRAS40 from Raptor, AKT also affects mTOR in a TSC-independent fashion^{4,5}.

Amino acids regulate mTORC1 via Ras-related GTPase (Rag) proteins, of which there are four (RagA to RagD). The presence of amino acids triggers the formation of an active state (RagA/B(GTP)-RagC/D(GDP)) which then binds to Raptor and translocates mTORC1 to the lysosomal surface, where Rheb is located, via the Ragulator complex⁹. Both Rag and Rheb are required for mTORC1 activation, meaning that growth factor and amino acid regulation of mTOR are inter-linked⁵.

Several cellular stressors act through TSC signalling to also affect mTORC1, including low ATP levels, hypoxia and DNA damage⁵. Glucose deprivation activates adenosine monophosphate-activated protein kinase (AMPK), which in turn activates TSC2 to inhibit mTORC1, as well as directly phosphorylating Raptor⁵. Hypoxia leads to the activation of TSC2 by inducing expression of transcriptional regulation of the DNA damage response 1 (REDD1 or RTP801¹⁰). Finally, DNA damage negatively regulates mTORC1 through TSC2, AMPK, as well as phosphatase and tensin homolog deleted on chromosome 10 (PTEN)⁴. mTORC2, on the other hand, is insensitive to nutrients but responds to insulin/PI3K signalling, and is also affected by mTORC1 by way of a PI3K negative feedback loop⁵.

Figure 1: Examples of mTOR signalling pathways and cellular effects ^{4,5,11,12}



1.4. mTOR in cellular growth and proliferation

mTOR is the main nutrient-sensitive mediator of growth in animals¹², sensing nutrient availability and promoting protein synthesis and cellular growth when energy and nutrient conditions are favourable. mTORC1 mediates protein synthesis by phosphorylating two regulators of mRNA translation: S6 kinase 1 (S6K1) and

eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1)⁵, which activate multiple effectors of mRNA translation and contribute to ribosomal assembly, respectively^{4,5}.

In addition to protein synthesis, mTORC1 also mediates lipid synthesis through sterol regulatory element-binding protein (SREBP) transcription factor⁶. Furthermore, mTORC1 activates the translation of hypoxia inducible factor 1 α (HIF-1 α), which promotes glycolytic flux^{4,5} and is a key transcription factor for vascular endothelial growth factor (VEGF)¹³. Figure 1 summarises some of the main regulators and effectors of the mTOR signalling pathway.

1.5. mTOR and development

mTOR signalling is critical for neurogenesis and is therefore pivotal for normal neurological development. It serves important roles in stem cell differentiation, neuronal structure, dendritic morphogenesis and axon outgrowth³. The activity of RTP801, an endogenous negative regulator of mTOR, decreases as development progresses, and thus enhanced mTOR function promotes the maturation of neuronal progenitor and newborn neurons¹⁴. Dysfunction of the mTOR pathway may be associated with neurodevelopmental disorders, such as tuberous sclerosis¹⁵.

The mTOR pathway regulates neurogenesis in the eye and precise spatiotemporal regulation of pathway activity is central to normal development of the retina and optic nerve¹⁶. mTOR pathway activity is temporally associated with retinal ganglion cell (RGC) differentiation in rat and human models¹⁷. Furthermore, studies of the *Drosophila* retina have shown that experimental mTOR pathway activation causes premature differentiation of photoreceptors, while mTOR inhibition delays differentiation¹⁶. Similarly, mouse models of mTORC1 hyperactivation have demonstrated precocious development of retinal cells that can be arrested by

rapamycin¹⁸. The loss of mTOR signalling induced by experimental deletion of Raptor or rictor in mouse models has been associated with abnormalities in cone outer and inner segment morphology¹⁹. Curiously, the loss of either mTORC1 or mTORC2 alone in knockout mice does not appear to affect cone function on electroretinography (ERG) at 1 year of age, however significant functional losses are evident upon combined loss of mTORC1 and mTORC2¹⁹. mTOR also plays a role in vascular development via HIF-1 α mediated VEGF expression, and disruption of this pathway through mTORC1 inhibition has been shown to delay, but not completely suppress models of retinal vascularisation²⁰.

1.6. mTOR and autophagy

The mTOR pathway plays an important role in regulation of autophagy, chiefly via mTORC1 mediated suppression of macroautophagy. Macroautophagy is a cellular recycling process in which proteins and organelles are degraded, yielding substrates for reuse and removing dysfunctional cellular constituents. This process begins with phagophore formation, mediated by proteins of the UNC51-like kinase (ULK) complex (including ULK1). The material to be phagocytosed is encompassed by a double membrane structure, known as an autophagosome, which then fuses with a lysosome, degrades the material and mobilises substrates for reuse in the cell. Thus, in response to a diverse array of stresses – including starvation, hypoxia and infection – macroautophagy recycles a wide array of resources including carbohydrates, lipids and minerals for redeployment in cells²¹. Impairment of autophagy, for instance through sustained mTORC1 activation, results in the loss of a quality control mechanism by which misfolded proteins and dysfunctional organelles can be removed. Autophagic dysfunction has been implicated in a number of neurodegenerative diseases, including Alzheimer's disease and Parkinson's

disease, in which protein aggregation is a key feature¹⁵. Accordingly, inhibitors of mTORC1, such as rapamycin, have therapeutic potential in these diseases¹⁵.

Autophagy is essential for ocular health. For example, retinal pigment epithelium (RPE) cells are post-mitotic and have limited renewal capacity, but are protected from the toxic effects of accumulated proteins by a high basal rate of autophagy in healthy retinas^{22,23}. In vitro studies of ethanol toxicity on a human RPE cell line (ARPE-19 cells) have demonstrated that inhibition of autophagic clearance leads to increased cell death, as autophagy plays a protective role by removing fragmented mitochondria²⁴.

By the same token, pro-survival autophagic processes are upregulated in retinal ganglion cells (RGCs) in response to stress, including in studies of optic nerve crush or experimental intraocular pressure (IOP) elevation^{22,23}.

Autophagy is thus of great interest in the understanding and treatment of several ophthalmic conditions including age-related macular degeneration (AMD), diabetic retinopathy (DR), glaucoma and retinal dystrophies²². Many of these insights were obtained in studies involving modulation of the mTOR pathway, particularly with rapamycin. Advances in our understanding of autophagy and the development of therapies to selectively modulate autophagic pathways could offer great therapeutic potential for a wide range of diseases²².

1.7. mTOR in immune function

The mTOR pathway plays a complex role in the immune system and has a broad set of influences on lymphocyte function and metabolism. mTORC1 and mTORC2 signalling impact helper T-cell (Th1, Th2, Th17 and Tfh) differentiation, and mTORC1 appears to be critical for regulatory T-cell (Treg) function^{5,25}. Rapamycin (sirolimus) has immunosuppressant activity via the inhibition of mTORC1 and thus T-

cell inactivation, and it was approved in 1999 by the US Food and Drug Administration as a drug for use in renal transplant recipients^{2,5}. Furthermore, rapamycin has additional anti-inflammatory activities via the inhibition of cyclooxygenase-2 (COX-2) mRNA expression and prostaglandin E2 (PGE2) production²⁶. Accordingly, there has been interest in the potential of rapamycin as an immunosuppressant in ophthalmic conditions, particularly in non-infectious uveitis, given the central role of helper T-cell activation and clonal expansion of Th cells in the disease²⁷. A number of preclinical studies of the drug have highlighted the potential of intravitreal rapamycin in non-infectious uveitis and human clinical trials of the drug are underway^{2,27,28}. The immunological effects of mTOR inhibitors have also been of interest for a range of other diseases including AMD²⁶ and DR²⁹, as discussed below (see Table 1).

1.8. mTOR and ageing

Rapamycin is one of a small number of therapies that have been demonstrated to extend the lifespan of organisms across a wide phylogenetic range – from yeast (*Saccharomyces cerevisiae*), to nematodes (*Caenorhabditis elegans*), flies (*Drosophila melanogaster*) and even mammals (*Mus musculus*) – and this effect is considered to be mediated via mTOR. Given the conservation of the pathway in these species, the influence of the mTOR on longevity is likely to extend to humans as well⁵. However, the mechanisms by which mTOR influences ageing are complex and not yet fully elucidated. Key candidate effects include those on the regulation of mRNA translation, the activation of autophagy and the downregulation of inflammation.

Ageing is an important factor in a range of eye diseases and accordingly, mTOR signalling is likely to play a role in these diseases. For example, studies of aged RPE

cells have demonstrated increased mTORC1 activity and recruitment to the lysosomal surface, and this has been associated with a reduced capacity of the cells to phagocytose shed photoreceptor outer segments³⁰. The phagocytic function of RPE cells is integral to retinal health and dysfunction of the process has been implicated in the pathogenesis of AMD³¹.

1.9. mTOR and cancer

The mTOR signalling network is implicated in the deregulation of many oncogenic pathways, chiefly via mTOR hyperactivation due to pathway activating mutations or the loss of inhibitory regulators³². In some cancers mTOR is constitutively activated and independent of environmental stimuli and this favours tumour growth⁷. Indeed the PI3K/AKT/mTOR pathway is one of the most frequently activated pathways in cancer and is considered to play a central role in some 30-50% of tumours³³. Accordingly, two rapamycin derivatives (everolimus and temsirolimus) are FDA approved therapies for cancer, and several second-generation ATP-competitive mTOR inhibitors and a number of dual PI3K/mTOR inhibitors are in development³⁴.

Interestingly, the mammalian retina shares a special metabolic phenomenon of aerobic glycolysis with neoplastic cells. This is known as the Warburg effect, and is partially mediated by HIF-1 α , which is itself regulated by mTORC1¹¹. Aerobic glycolysis promotes a “metabolic budget system” that sustains the energy demands of proliferating tumour cells. Similarly, in the retina, the Warburg effect is thought to sustain the biosynthetic demands of constant photoreceptor outer segment turnover³⁵. The ramifications of mTOR inhibition on this effect have not yet been elucidated.

2. MTOR IN RETINAL DISEASES

The centrality of the mTOR pathway to cell growth, metabolism, autophagy and immune function means that dysregulation of the pathway is important in a range of retinal diseases including, but not limited to AMD, DR and retinitis pigmentosa (RP).

Table 1: Summary of clinical trials of mTOR inhibitors in retinal disease

Drug	Condition	Evidence
Sirolimus (rapamycin)	Non-infectious Uveitis	Phase III trials of 440µg intravitreal injection – trials ongoing ²⁷
	AMD (GA)	Phase II trial of 3-monthly 440µg subconjunctival injection (n=8), vs untreated fellow eye – no benefit ³⁶
		Phase II trial of monthly 440µg intravitreal injection (n=27), vs sham – no benefit ³⁷
	AMD (CNV)	Phase II trial of second-daily 2mg oral tablet (n=3), vs daclizumab, vs infliximab, vs no immunosuppression – no benefit ³⁸
		Phase II trial of 2-monthly 440µg intravitreal injection plus aflibercept (n=10), vs aflibercept alone – awaiting results ³⁹
		Phase II trial of 2-monthly intravitreal injection (n=18), vs anti-VEGF therapy – awaiting results ⁴⁰
DMO	Phase I/II trial of 440µg subconjunctival injection (n=5), vs fellow eye – appears safe, efficacy studies required ⁴¹	
RES-529 (Palomid 529)	AMD (CNV)	Phase I trial of monthly 1.9mg subconjunctival injection (n=5) – appears safe, efficacy studies required ⁴²

2.1. Non-exudative AMD and mTOR

AMD is a complex disease with multiple pathogenic mechanisms, many of which intersect with the mTOR pathway – namely factors such as ageing, autophagy and immune function. Indeed, robust mTOR pathway activation can be detected in multiple models of AMD, and treatment with rapamycin has a protective effect and ameliorates AMD-like pathology^{43,44,45}. In AMD, RPE dysfunction and degeneration are hallmarks of AMD which precede geographic atrophy (GA) and choroidal neovascularisation (CNV)⁴⁶. RPE forms part of the protective blood-retinal-barrier, which contributes to retinal immune privilege and allows nutritional support to be transported to photoreceptors³¹. The RPE is also responsible for phagocytosis and degradation of shed photoreceptor outer segments and recycling of molecules that support the visual cycle. Ageing is associated with the progressive accumulation of lipofuscin in the cytoplasm of RPE cells which inhibits lysosomal activity and autophagy⁴⁷. In vitro studies have shown that a major component of lipofuscin, N-retinyl-N-retinylidene ethanolamine (A2E), has a detrimental effect on RPE and upregulates inflammatory factors as well as VEGF-A⁴³.

Inflammation, another factor that can be modulated by mTOR pathways, may also play an important role in AMD pathogenesis. Chronic inflammation can contribute to the formation of drusen, extracellular subretinal deposits, which are themselves likely pro-inflammatory⁴⁸. RPE degeneration is thought to foster a pro-inflammatory microenvironment, characterised by inflammatory cell infiltration and a dysregulated parainflammatory response^{26,49}. The effects of the mTOR pathway therefore overlap with several pathogenic processes in AMD. Accordingly, the pro-autophagic and immunosuppressive actions of mTOR inhibitors make them attractive candidates for the treatment of AMD.

2.2. Non-exudative AMD – clinical trials of mTOR modulators

Interventional studies targeting the mTOR pathway in non-exudative AMD have largely focussed on the use of rapamycin in late stage disease. Wong et al. studied subconjunctival rapamycin (sirolimus) in delaying the progression of GA in a small open-label phase II trial. Although the treatment was well tolerated, no significant differences were observed in the progression of GA between 8 treated and fellow 8 untreated control eyes, however visual acuity loss was greater in treated eyes³⁶. A follow-up study of intravitreal rapamycin by the same centre was suspended due to ocular adverse events, including increased paralesional fundus autofluorescence abnormalities and central retinal thinning⁵⁰. A multicentre, randomised controlled trial of intravitreal rapamycin in GA was conducted as an ancillary study to the Age Related Eye Disease Study 2 (AREDS2). 52 participants were randomised to receive either rapamycin or sham injection on a monthly basis. The trial was suspended due to ocular adverse effects, after 3 participants in the rapamycin arm developed sterile endophthalmitis. No significant differences were found for changes in GA area, central subfoveal thickness or visual acuity loss between the two groups³⁷.

The lack of benefit of rapamycin for GA may have several explanations. For one, although inflammation is implicated in the pathogenesis of AMD, the effects of immunosuppressive and anti-inflammatory therapies may be insufficient alone to halt progression of the disease in its late stages^{36,37}. Likewise, although the upregulation of autophagy may have protective effects in early AMD, in advanced disease increased autophagy could be detrimental due to the loss of vulnerable cells⁴⁶. Finally, as is true for other complex signalling pathways, the effects of therapeutic modulation are often unpredictable. For example, whilst mTORC2 is relatively insensitive to rapamycin, long term use of the drug may inhibit the complex and lead to unintended effects⁵¹. Furthermore, the mTOR pathway may have cell-type and context-specific effects, such that modulation of the pathway may

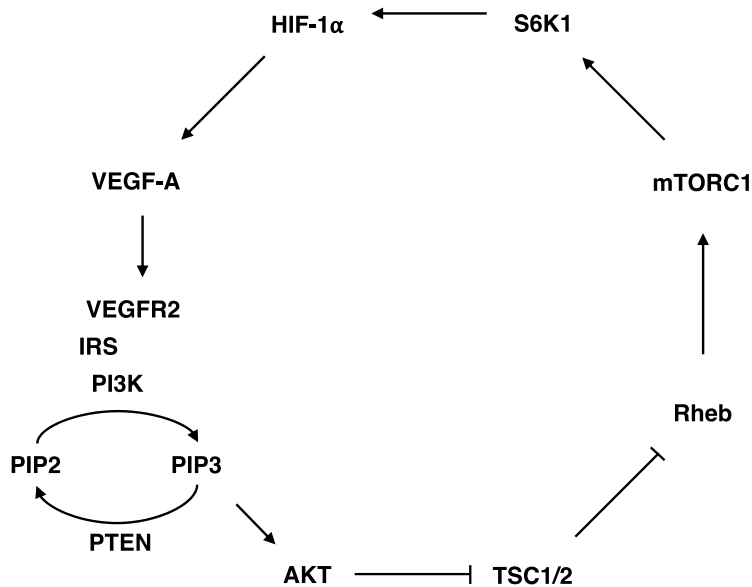
result in seemingly paradoxical outcomes. In the lung, for instance, rapamycin has been shown to be anti-inflammatory in some contexts and pro-inflammatory in others⁵². In the eye rapamycin has been observed to suppresses uveitic inflammation in some pre-clinical models and exacerbate it in others². Much is still unknown about the pathway, particularly as it pertains to the early stages of AMD and, more broadly, about the full range of consequences of mTOR inhibition. Nevertheless, clinical trials of mTOR modulation in CNV have generated more encouraging results.

2.3. Exudative AMD and mTOR

The formation of CNV, a process central to neovascular AMD, is thought to be driven in part by RPE stress and the release of pro-inflammatory and pro-angiogenic factors such as VEGF-A⁴⁶. VEGF-A is key effector of ocular angiogenic disorders, underpinning the choroidal neovascularisation that occurs in AMD and other disorders, as well as the retinal neovascularisation in proliferative DR and retinal vein occlusion (RVO)⁵³. VEGF-A orchestrates an angiogenic cascade characterised by increased vascular permeability, endothelial cell activation, degradation of basement membranes and the proliferation and migration of endothelial cells.

The mTOR pathway plays a role in this process via the activation of HIF-1 α , which then induces the transcription of VEGF-A. This activates VEGFR-1 and VEGFR-2 to increase vascular permeability and endothelial cell sprouting from existing blood vessels^{4,53,54}. VEGFR-2 signalling activates PI3K which, in turn, exerts a downstream activating effect on mTOR. mTOR thus serves as a fulcrum for a self-amplifying autocrine loop of VEGF signalling^{8,54,55,56} (See Fig 2).

Figure 2: Autocrine loop of mTOR-VEGF signalling^{4,55,57}



mTOR inhibition not only blocks HIF-1 α , thus inhibiting an upstream regulator of VEGF, but it also inhibits the endothelial response to VEGF¹³, and reduces VEGF-mediated endothelial cell proliferation and migration⁵⁸. Accordingly, there has been considerable interest in mTOR inhibitors as potential therapies for retinal and choroidal neovascularisation.

The laser trauma model is a classic experimental model for CNV that uses laser energy to rupture Bruch's membrane and allow the invasion of choroidal vessels into the subretinal space⁵⁹. In mouse models of laser induced CNV, HIF-1 α and VEGF upregulation are accompanied by increases in mTORC1 and mTORC2 activity. Interestingly, mTORC1 activity appears to be most prominent in inflammatory cells whereas mTORC2 is most active in vascular endothelial cells and pericytes⁵⁹. Rapamycin has been demonstrated to inhibit experimental CNV in this model⁵⁹. In another study, the intravitreal injection of recombinant adeno-associated virus (rAAV) expressing short hairpin RNA (shRNA) targeting mTORC1 and mTORC2 reduced CNV area and inflammatory cell infiltration as well as increasing autophagy⁶⁰.

The current standard of care for CNV and many ocular angiogenic disorders is anti-VEGF therapy⁵³. Accordingly, mTOR inhibition has been compared with anti-VEGF monotherapy in a number of preclinical studies^{61,62}. In one study rapamycin was shown to be more effective at suppressing in vitro sprouting of human umbilical vein endothelial cells (HUVEC), when co-cultured with RPE cells, than the VEGF inhibitors bevacizumab and ranibizumab⁶¹.

Omipalisib, a dual PI3K/mTOR inhibitor currently undergoing trials for the systemic treatment of cancer and idiopathic pulmonary fibrosis, has also been evaluated for CNV³⁴. In laser-induced CNV mouse models, intravitreal omipalisib (GSK2126458) has been demonstrated to outperform the VEGF inhibitor aflibercept. Oral omipalisib has also been shown to inhibit CNV in the same model but is associated with a systemic diabetogenic effect, probably through blockade of insulin signalling⁶².

2.4. Exudative AMD – clinical trials of mTOR modulators

Human serum biomarker studies have implicated the mTOR pathway in CNV. A study of circulating microRNA (cmiRNA), small noncoding RNAs involved in post-transcriptional gene expression regulation⁶³, found differences in the expression of three cmiRNAs between people with CNV and healthy volunteers. Pathway enrichment analysis of genes likely to be regulated by these cmiRNAs identified the mTOR and Transforming Growth Factor- β (TGF- β) pathways⁶⁴.

A range of early phase human clinical trials have tested mTOR modulators for CNV both as monotherapies and in combination with anti-VEGF agents. One pilot study of oral rapamycin (sirolimus) in 3 patients with active CNV receiving intravitreal anti-VEGF therapy sought to identify whether rapamycin reduced the need for anti-VEGF injections³⁸. No significant differences were found between the groups for injection

frequency, BCVA or OCT parameters after 6 months of treatment³⁸. A recent conference proceeding indicated that intravitreal rapamycin in combination with aflibercept was more effective than aflibercept alone for intraretinal and subretinal fluid reduction in patients with active CNV^{39,65}. A phase 2 randomised head-to-head comparison of intravitreal rapamycin monotherapy and anti-VEGF therapy was conducted by the same group and results are awaited^{40,66}.

A phase 1 trial of a dual inhibitor of mTORC1 and mTORC2, RES-529 (Palomid 529), in patients with persistent CNV on anti-VEGF therapy was recently reported⁴². The treatment was well tolerated in this pilot study. Whilst no beneficial effects were found for secondary outcome measures, the study was not adequately powered for these⁴². Given evidence from preclinical models of the roles of mTORC1 and mTORC2 in neovascularisation⁸, there is a robust rationale for dual inhibition to treat CNV.

Nevertheless, several mTOR inhibitor trials for CNV have been terminated by the trial sponsors. These include: intravitreal sirolimus versus subconjunctival sirolimus (NCT00712491); subconjunctival sirolimus and ranibizumab versus placebo and ranibizumab (NCT00766337); oral everolimus versus ranibizumab versus combination therapy (NCT00857259)⁶⁷.

In contrast to therapies that inhibit mTOR, some experimental CNV treatments may lead to off-target activation of the pathway. RTP801 is a stress-related protein which is upregulated by hypoxia and which, amongst other things, inhibits mTOR activity via TSC2^{10,52}. Therefore, inhibitors of RTP801 activate mTOR^{52,68}. One example is PF-655, a small interfering RNA (siRNA) that blocks the gene encoding RTP801 and it has been studied in CNV⁶⁹ and diabetic macular oedema (DMO)⁷⁰. In the MONET trial, 151 participants with active CNV were randomised to one of three treatments: PF-655 monotherapy, versus monthly intravitreal PF-665 plus ranibizumab, or monthly ranibizumab monotherapy⁶⁹. PF-655 monotherapy was inferior to

ranibizumab monotherapy and outcomes for those on combined treatment were comparable to those receiving ranibizumab monotherapy⁶⁹. It is not known whether off-target mTOR activation contributed to the negative results of the trial.

2.5. Diabetic retinopathy and mTOR

The pathogenesis of diabetic retinopathy (DR) is complex and multifactorial. Chronic hyperglycaemia induces dysregulation of multiple biochemical pathways, leading to oxidative stress that feeds into a cycle of further dysfunction and reactive oxygen species (ROS) production. This leads to a significant rise in inflammatory cytokines and hypoxia-driven VEGF secretion. Together with mitochondrial dysfunction, vascular and neural cell apoptosis and aberrant function of the neurovascular unit, the clinical disease progressively manifests as pericyte loss, microaneurysms, haemorrhages, capillary non-perfusion, leakage and neovascularisation^{29,53}.

mTOR signalling plays a diverse array of roles in the pathogenesis of diabetes and its complications, including retinopathy. Sustained hyperactivation of mTORC1 drives pancreatic β -cell failure and peripheral insulin resistance, promoting the progression of Type 2 diabetes (T2DM) pathology⁴. Hyperglycaemia leads to the activation of mTORC1 via the inhibition of AMPK as well as PI3K activation⁵⁴. A state of nutrient excess may also promote mTORC1 activation, compounding the cycle⁴. Rats fed a pro-diabetogenic diet demonstrate altered responses on ERG, and analysis of retinal RNA expression shows significant perturbation of the mTOR pathway⁷¹. The contribution of mTOR to DR pathological processes is likely multifactorial and probably encompasses its roles in immune function, endothelial cell proliferation, VEGF-signalling and autophagy regulation.

The transcription factor nuclear factor kappa-B (NF- κ B) is activated by hyperglycaemia and causes pericyte apoptosis, a hallmark of DR, and also induces a

pro-inflammatory response that is linked with mTOR activity⁵⁴. Proliferating endothelial cells also show mTOR activation in mouse models of retinal angiogenesis, which is blocked by both rapamycin and everolimus⁵⁶. VEGF-mediated proliferation and migration of RPE cells and vascular endothelial cells in culture is likewise inhibited by temsirolimus⁵⁸.

As previously mentioned, the mTOR pathway plays an important role in the hypoxic induction of VEGF expression and this is a key pathogenic event in DR⁵⁴. In mouse models of induced hyperglycaemia, increased expression of HIF-1 α , VEGF-A and downstream effects of mTOR signalling are attenuated by intravitreal rapamycin⁷². Hyperglycaemia-induced dysfunction of Müller cells is associated with the expression of VEGF and inflammatory cytokines⁷³. Laboratory studies also demonstrate that high glucose triggers dysfunctional autophagy in Müller cells that can further exacerbate VEGF release and cause cell death⁷⁴. mTOR inhibition with rapamycin protects Müller cells through restoration of autophagy, reducing VEGF release and downregulating apoptosis⁷⁴. Conversely, experimental ablation of Müller cells in a transgenic mouse model showed localised suppression of mTOR in photoreceptor inner and outer segments, possibly as a consequence of photoreceptor degeneration in areas of Müller cell loss⁷⁵.

2.6. Diabetic macular oedema – clinical trials of mTOR modulators

Given promising findings in pre-clinical studies, mTOR modulators have been studied in phase 1/2 human clinical trials for diabetic macular oedema with disappointing results. A small prospective trial of 8-weekly subconjunctival rapamycin (sirolimus) for 12 months, provided limited evidence of a treatment response relative to untreated fellow eyes⁴¹. The authors also reported that a larger randomised trial at another centre had been stopped due to lack of efficacy. Phase 1 study data suggest that locally administered sirolimus is safe⁷⁶; this is particularly important in this

population as oral treatment can be associated with hyperlipidaemia¹⁵. Furthermore, although systemic mTORC1 activation can increase hyperglycaemia, prolonged rapamycin treatment can inhibit mTORC2, and lead to insulin resistance⁵.

Intravitreal PF-655, the same inhibitor of RTP801 as was used in the MONET study for AMD, was studied as a candidate therapy for DMO in the DEGAS trial. 184 participants with DMO were randomised to receive one PF-655 or macular (focal/grid) photocoagulation. The study was terminated prematurely due to lack of efficacy and adverse effects⁷⁰. As was the case for the MONET trial, it is unclear whether the off-target activation of mTOR was contributory to this failure.

2.7. Retinitis pigmentosa

RP is a collection of inherited retinal dystrophies that leads to the progressive loss of photoreceptors and RPE. Mutations in more than 80 genes have been implicated in the pathogenesis of RP, making it a clinically and pathologically heterogeneous condition⁷⁷. RP mutations primarily cause the degeneration and cell death of rod photoreceptors; until the loss of rods is extensive, cone function may remain relatively intact. Thereafter the progressive death of cone photoreceptors causes the loss of visual acuity. Multiple pathogenic mechanisms have been proposed for various subtypes of the disease, but it appears that metabolic dysfunction linked to mTOR may contribute to cone death⁷⁸.

Punzo et al. studied four RP mouse models at multiple time points during rod and cone degeneration and found that a decline in phosphorylated mTOR coincided with cone degeneration⁷⁹. More recently, PTEN, a negative regulator of mTOR, was found to be upregulated at a critical stage of photoreceptor loss⁸⁰. Constitutive activation of mTORC1 in cones via disruption of PTEN or TSC led to improved cell metabolism

and survival in two models of RP for up to 8 months⁸¹. Furthermore, TSC1 knockout in rods, and thus upregulation of mTOR, resulted in improved survival and function of rods as well as cones⁸².

These studies suggest that mTOR modulation could have potential as a mutation-independent therapeutic avenue for RP, but this is not without its challenges. TSC ablation carries the risk of cancer, although no tumours were detected at 16 months in TSC1 knockout mice⁸². Secondly, there was a loss of pro-survival effect over time due to impaired autophagy. Ironically, rapamycin treatment was protective in these mice via the restoration of autophagy⁸³. Another treatment strategy has been to target AKT, which negatively regulates the suppressive TSC2 and thus activates mTORC1. Specifically, AKT3 has been targeted due to its apparent neurodevelopmental importance and potent stimulation of retinal mTORC1. Subretinal injection of rAAV-AKT3 showed an initial protective effect for photoreceptors in RP-model mice however functional gains were shortlived⁸⁴. Targeting the downstream mTORC1 effector S6K1 is another potential treatment option. Subretinal injection of a viral vector (rAAV-S6K1) to overexpress S6K1 in the cones of a RP mouse model led to improved photoreceptor survival compared to control injection. Furthermore, functional improvement on ERG remained statistically significant at post-natal day 40, as did improvement visual behaviour at post-natal day 26⁸⁰. Further study is needed before this and other strategies targeting mTOR are translated to the clinic for people with RP.

3. MTOR IN DISEASES OF THE OPTIC NERVE

The mTOR signalling network is important for neurogenesis and neuronal health and thus the health of the optic nerve. Optic nerve trauma is well-studied as a classic experimental model of CNS injury, as the limited innate regenerative ability of retinal

ganglion cells is typical of neurons elsewhere in the CNS⁵⁷. Through these models, insights about the mTOR pathway in conditions such as traumatic optic neuropathy and glaucoma can be ascertained.

3.1 Traumatic optic neuropathy

Animal models of traumatic optic neuropathy show rapid and sustained degeneration of RGCs following injury. The rat model of intraorbital optic nerve axotomy is accompanied by approximately 50% RGC degeneration at 4 days, 70% after one week and 95% after 2 weeks⁸⁵. Likewise, the severe crush injury model shows approximately 50% degeneration after 1 week, 70% after 2 weeks and 90% after 8 weeks⁸⁵. Following axon injury, analysis of the mTOR pathway shows a rapid downregulation in activity in RGCs that precedes cell loss⁸⁶. The α RGC subtype survives preferentially and has high endogenous mTOR activity⁸⁷. Modulation of the mTOR pathway thus holds promise for the neuroprotection and regeneration of injured RGCs⁵⁷. The upregulation of mTOR by TSC2-shRNA, for instance, significantly improves regeneration after axotomy compared to control, an effect that is blocked by rapamycin¹⁷. Furthermore, inhibitory RTP801 is upregulated after axotomy and likely contributes to the injury-related suppression of mTOR activity. Intravitreal RTP801-siRNA restores mTOR activity and improves RGC survival after optic nerve crush to 82% at 24 days, compared with 45% for controls at the same time point ($p < 0.001$)^{68,86}. Another strategy is PTEN deletion which, especially when combined with SOCS3 deletion, improves axon regeneration in part through its effect on mTOR⁸⁸. mTOR activation also likely contributes to the axon regenerating effect of post-injury inflammation⁸⁹.

However, the ubiquity of the mTOR pathways makes its role in traumatic optic neuropathy highly complex. For one, autophagy appears to be cytoprotective in acute optic nerve injury²². In axotomised mice additionally stressed with paraquat to

induce reactive oxygen species, rapamycin plays a protective role rather than being detrimental⁹⁰. At present there are no published reports of the use of mTOR-modulating treatments for traumatic optic neuropathy in humans.

3.2. Glaucoma

The animal models of optic nerve trauma have been extrapolated to glaucoma research⁹⁰. mTOR has also been investigated in models of elevated intraocular pressure, but studies have produced varying results depending on the mechanism of injury, as well as the cell type and time course studied^{91,92}. A recent rat study of experimental glaucoma induced by laser of the trabecular meshwork (TM) and episcleral veins found activation of mTOR as well as S6K1 and 4E-BP1, in addition to elevated HIF-1 α and VEGF in retinal tissue⁹¹. However, a separate rat study of episcleral vein cauterization found that retinal mTOR was downregulated after 1 week post-injury⁹².

Interestingly, although rapamycin treatment does not appear to exert a significant effect on IOP^{91,93,94}, it does appear to offer a measure of neuroprotection against axonal degeneration in TM laser models⁹³. Enhanced RGC survival has been demonstrated with rapamycin treatment following episcleral vein cauterization and this has been associated with reduced microglial activation and reactive oxygen species levels⁹⁴. The beneficial effects of increased autophagy in experimental glaucoma are less clear as there are reports that this may be detrimental in the acute setting^{92,95}. Downstream mTOR signalling has been shown to be disrupted in human glaucomatous TM compared to non-glaucomatous TM in vitro⁹⁶, and there is evidence that rapamycin is protective against rotenone-induced TM oxidative stress⁹⁷. However, a case series of human volunteers with ocular hypertension,

normotensive glaucoma and healthy controls found no significant differences in mTOR signalling in the bloodstream⁹⁸.

The role of mTOR and its modulation in glaucoma thus appears to be complex, likely in part due to the multifactorial nature of the condition and in part to the multiple and complex actions of the pathway.

4. CONCLUSION

Major advances in our understanding of the mTOR pathway have been made in the 50 years since its discovery. These advances have underscored both the ubiquity and complexity of the pathway and it is apparent that it regulates many aspects of systemic and ocular health and disease. The central role of the pathway in many diseases makes it both an appealing and a challenging therapeutic target, as although drugs modulating the pathway may have great potential, they are also highly likely to have a wide range of adverse effects. The development of newer modulators of mTOR signalling are likely to reveal further insights into the pathway that will inform our understanding of diseases of the retina and optic nerve and ultimately new treatments for them.

REFERENCES

1. Watve MG, Tickoo R, Jog MM, Bhole BD. How many antibiotics are produced by the genus *Streptomyces*? *Arch Microbiol.* 2001;176(5):386-90.
2. Agarwal A, Rajagopalan N, Hassan M, Sadiq MA, Soliman MK, Afridi R, et al. Sirolimus for Retinal and Uveitic Diseases. In: Nguyen QD, Rodrigues EB, Farah ME, Mieler WF, Do DV, editors. *Retinal Pharmacotherapeutics. Developments in Ophthalmology.* 55. Basel: Karger; 2016. p. 276-81.
3. Maiese K. Targeting molecules to medicine with mTOR, autophagy and neurodegenerative disorders. *Br J Clin Pharmacol.* 2016;82(5):1245-66.
4. Laplante M, Sabatini David M. mTOR Signaling in Growth Control and Disease. *Cell.* 2012;149(2):274-93.
5. Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. *Cell.* 2017;168(6):960-76.
6. Kim J, Guan KL. mTOR as a central hub of nutrient signalling and cell growth. *Nat Cell Biol.* 2019;21(1):63-71.
7. El Hiani Y, Egom EE, Dong XP. mTOR signalling: jack-of-all-trades. *Biochem Cell Biol.* 2019;97(1):58-67.
8. Yang JY, Madrakhimov SB, Ahn DH, Chang HS, Jung SJ, Nah SK, et al. mTORC1 and mTORC2 are differentially engaged in the development of laser-induced CNV. *Cell Commun Signal.* 2019;17:14.
9. Kim J, Kim E. Rag GTPase in amino acid signaling. *Amino Acids.* 2016;48(4):915-28.
10. Rittenhouse KD, Johnson TR, Vicini P, Hiraakawa B, Kalabat D, Yang AH, et al. RTP801 Gene Expression Is Differentially Upregulated in Retinopathy and Is Silenced by PF-04523655, a 19-Mer siRNA Directed Against RTP801. *Investigative Ophthalmology & Visual Science.* 2014;55(3):1232-40.

11. Johnson SC, Sangesland M, Kaeberlein M, Rabinovitch PS. Modulating mTOR in Aging and Health. In: Yashin AI, Jazwinski SM, editors. Aging and Health - a Systems Biology Perspective. Interdisciplinary Topics in Gerontology and Geriatrics. 402015. p. 107-27.
12. Sabatini DM. Twenty-five years of mTOR: Uncovering the link from nutrients to growth. *Proceedings of the National Academy of Sciences*. 2017;114(45):11818-25.
13. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, et al. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat Med*. 2002;8(2):128-35.
14. Malagelada C, Lopez-Toledano MA, Willett RT, Jin ZH, Shelanski ML, Greene LA. RTP801/REDD1 Regulates the Timing of Cortical Neurogenesis and Neuron Migration. *Journal of Neuroscience*. 2011;31(9):3186-96.
15. Crino PB. The mTOR signalling cascade: paving new roads to cure neurological disease. *Nat Rev Neurol*. 2016;12(7):379-92.
16. Avet-Rochex A, Carvajal N, Christoforou CP, Yeung K, Maierbrugger KT, Hobbs C, et al. Unkempt is negatively regulated by mTOR and uncouples neuronal differentiation from growth control. *PLoS Genet*. 2014;10(9):e1004624.
17. Teotia P, Van Hook MJ, Fischer D, Ahmad I. Human retinal ganglion cell axon regeneration by recapitulating developmental mechanisms: effects of recruitment of the mTOR pathway. *Development*. 2019;146(13).
18. Choi J-H, Jo HS, Lim S, Kim H-T, Lee KW, Moon KH, et al. mTORC1 accelerates retinal development via the immunoproteasome. *Nature Communications*. 2018;9(1):2502.
19. Ma S, Venkatesh A, Langellotto F, Le YZ, Hall MN, Ruegg MA, et al. Loss of mTOR signaling affects cone function, cone structure and expression of cone specific proteins without affecting cone survival. *Exp Eye Res*. 2015;135:1-13.

20. Yagasaki R, Nakahara T, Mori A, Sakamoto K, Ishii K. Effects of mTOR inhibition on normal retinal vascular development in the mouse. *Experimental Eye Research*. 2014;129:127-34.
21. Kaur J, Debnath J. Autophagy at the crossroads of catabolism and anabolism. *Nature Reviews Molecular Cell Biology*. 2015;16(8):461-72.
22. Boya P, Esteban-Martinez L, Serrano-Puebla A, Gomez-Sintes R, Villarejo-Zori B. Autophagy in the eye: Development, degeneration, and aging. *Prog Retin Eye Res*. 2016;55:206-45.
23. Lin W, Xu G. Autophagy: A Role in the Apoptosis, Survival, Inflammation, and Development of the Retina. *Ophthalmic Res*. 2019;61(2):65-72.
24. Bonet-Ponce L, Saez-Atienzar S, da Casa C, Flores-Bellver M, Barcia JM, Sancho-Pelluz J, et al. On the mechanism underlying ethanol-induced mitochondrial dynamic disruption and autophagy response. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2015;1852(7):1400-9.
25. Myers DR, Wheeler B, Roose JP. mTOR and other effector kinase signals that impact T cell function and activity. *Immunol Rev*. 2019;291(1):134-53.
26. Wang Y, Wang VM, Chan CC. The role of anti-inflammatory agents in age-related macular degeneration (AMD) treatment. *Eye (Lond)*. 2011;25(2):127-39.
27. Nguyen QD, Merrill PT, Sepah YJ, Ibrahim MA, Banker A, Leonardi A, et al. Intravitreal Sirolimus for the Treatment of Noninfectious Uveitis: Evolution through Preclinical and Clinical Studies. *Ophthalmology*. 2018;125(12):1984-93.
28. Cabahug VLO, Uy HS, Yu-Keh E, Sapno KJD. Outcomes of treatment with sirolimus for non-infectious uveitis: a meta-analysis and systematic review. *Clin Ophthalmol*. 2019;13:649-69.
29. Whitehead M, Wickremasinghe S, Osborne A, Van Wijngaarden P, Martin KR. Diabetic retinopathy: a complex pathophysiology requiring novel therapeutic strategies. *Expert Opin Biol Ther*. 2018;18(12):1257-70.

30. Yu B, Xu P, Zhao Z, Cai J, Sternberg P, Chen Y. Subcellular distribution and activity of mechanistic target of rapamycin in aged retinal pigment epithelium. *Invest Ophthalmol Vis Sci*. 2014;55(12):8638-50.
31. Strauss O. The Retinal Pigment Epithelium in Visual Function. *Physiological Reviews*. 2005;85(3):845-81.
32. Hua H, Kong Q, Zhang H, Wang J, Luo T, Jiang Y. Targeting mTOR for cancer therapy. *Journal of Hematology & Oncology*. 2019;12(1):71.
33. Bauer TM, Patel MR, Infante JR. Targeting PI3 kinase in cancer. *Pharmacology & Therapeutics*. 2015;146:53-60.
34. Elmenier FM, Lasheen DS, Abouzid KAM. Phosphatidylinositol 3 kinase (PI3K) inhibitors as new weapon to combat cancer. *Eur J Med Chem*. 2019;183:31.
35. Ng SK, Wood JPM, Chidlow G, Han GG, Kittipassorn T, Peet DJ, et al. Cancer-like metabolism of the mammalian retina. *Clinical and Experimental Ophthalmology*. 2015;43(4):367-76.
36. Wong WT, Dresner S, Forooghian F, Glaser T, Doss L, Zhou M, et al. Treatment of geographic atrophy with subconjunctival sirolimus: results of a phase I/II clinical trial. *Invest Ophthalmol Vis Sci*. 2013;54(4):2941-50.
37. Gensler G, Clemons TE, Domalpally A, Danis RP, Blodi B, Wells J, 3rd, et al. Treatment of Geographic Atrophy with Intravitreal Sirolimus: The Age-Related Eye Disease Study 2 Ancillary Study. *Ophthalmol Retina*. 2018;2(5):441-50.
38. Nussenblatt RB, Byrnes G, Sen HN, Yeh S, Faia L, Meyerle C, et al. A randomized pilot study of systemic immunosuppression in the treatment of age-related macular degeneration with choroidal neovascularization. *Retina*. 2010;30(10):1579-87.
39. Sirolimus in Conjunction With Eylea vs Eylea Alone for Exudative AMD (NCT02732899) Bethesda, MD: NIH US National Library of Medicine - ClinicalTrials.gov; 2018 [Available from: <https://clinicaltrials.gov/ct2/show/study/NCT02732899>].

40. Sirolimus Versus Anti-Vascular Endothelial Growth Factor (antiVEGF) for Wet AMD (NCT02357342) Bethesda, MD: NIH US National Library of Medicine - ClinicalTrials.gov; 2017 [Available from: <https://clinicaltrials.gov/ct2/show/NCT02357342>].
41. Krishnadev N, Forooghian F, Cukras C, Wong W, Saligan L, Chew EY, et al. Subconjunctival sirolimus in the treatment of diabetic macular edema. *Graefes Arch Clin Exp Ophthalmol*. 2011;249(11):1627-33.
42. Dalal M, Jacobs-EI N, Nicholson B, Tuo J, Chew E, Chan CC, et al. Subconjunctival Palomid 529 in the treatment of neovascular age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 2013;251(12):2705-9.
43. Zhang J, Bai Y, Huang L, Qi Y, Zhang Q, Li S, et al. Protective effect of autophagy on human retinal pigment epithelial cells against lipofuscin fluorophore A2E: implications for age-related macular degeneration. *Cell Death Dis*. 2015;6:e1972.
44. Zhao C, Yasumura D, Li XY, Matthes M, Lloyd M, Nielsen G, et al. mTOR-mediated dedifferentiation of the retinal pigment epithelium initiates photoreceptor degeneration in mice. *J Clin Invest*. 2011;121(1):369-83.
45. Kolosova NG, Muraleva NA, Zhdankina AA, Stefanova NA, Fursova AZ, Blagosklonny MV. Prevention of Age-Related Macular Degeneration-Like Retinopathy by Rapamycin in Rats. *Am J Pathol*. 2012;181(2):472-7.
46. Ambati J, Fowler B. Mechanisms of Age-Related Macular Degeneration. *Neuron*. 2012;75(1):26-39.
47. Ferrington DA, Sinha D, Kaarniranta K. Defects in retinal pigment epithelial cell proteolysis and the pathology associated with age-related macular degeneration. *Prog Retin Eye Res*. 2016;51:69-89.
48. Kauppinen A, Paterno JJ, Blasiak J, Salminen A, Kaarniranta K. Inflammation and its role in age-related macular degeneration. *Cell Mol Life Sci*. 2016;73(9):1765-86.

49. Chen M, Xu H. Parainflammation, chronic inflammation, and age-related macular degeneration. *J Leukoc Biol.* 2015;98(5):713-25.
50. Petrou PA, Cunningham D, Shimel K, Harrington M, Hammel K, Cukras CA, et al. Intravitreal sirolimus for the treatment of geographic atrophy: results of a phase I/II clinical trial. *Investigative ophthalmology & visual science.* 2014;56(1):330-8.
51. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, et al. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell.* 2006;22(2):159-68.
52. Yoshida T, Mett I, Bhunia AK, Bowman J, Perez M, Zhang L, et al. Rtp801, a suppressor of mTOR signaling, is an essential mediator of cigarette smoke-induced pulmonary injury and emphysema. *Nat Med.* 2010;16(7):767-73.
53. Patil R, Wong CW, Michelet F, Teo K, Ting D, Tsai A, et al. Angiogenesis-Based Therapies for Eye Diseases. In: Mehta JL, Mathur P, Dhalla NS, editors. *Biochemical Basis and Therapeutic Implications of Angiogenesis.* Cham: Springer International Publishing; 2017. p. 259-97.
54. Fernandes R, Reis F. Chapter 23 - mTOR in Diabetic Nephropathy and Retinopathy. In: Maiese K, editor. *Molecules to Medicine with mTOR.* Boston: Academic Press; 2016. p. 379-93.
55. Trinh XB, Tjalma WAA, Vermeulen PB, Van den Eynden G, Van der Auwera I, Van Laere SJ, et al. The VEGF pathway and the AKT/mTOR/p70S6K1 signalling pathway in human epithelial ovarian cancer. *British journal of cancer.* 2009;100(6):971-8.
56. Nakahara T, Morita A, Yagasaki R, Mori A, Sakamoto K. Mammalian Target of Rapamycin (mTOR) as a Potential Therapeutic Target in Pathological Ocular Angiogenesis. *Biol Pharm Bull.* 2017;40(12):2045-9.
57. Berry M, Ahmed Z, Morgan-Warren P, Fulton D, Logan A. Prospects for mTOR-mediated functional repair after central nervous system trauma. *Neurobiology of Disease.* 2016;85:99-110.

58. Liegl R, Koenig S, Siedlecki J, Haritoglou C, Kampik A, Kernt M. Temsirolimus inhibits proliferation and migration in retinal pigment epithelial and endothelial cells via mTOR inhibition and decreases VEGF and PDGF expression. *PLoS One*. 2014;9(2):e88203.
59. Pennesi ME, Neuringer M, Courtney RJ. Animal models of age related macular degeneration. *Mol Asp Med*. 2012;33(4):487-509.
60. Park TK, Lee SH, Choi JS, Nah SK, Kim HJ, Park HY, et al. Adeno-Associated Viral Vector-Mediated mTOR Inhibition by Short Hairpin RNA Suppresses Laser-Induced Choroidal Neovascularization. *Mol Ther Nucleic Acids*. 2017;8:26-35.
61. Stahl A, Paschek L, Martin G, Gross NJ, Feltgen N, Hansen LL, et al. Rapamycin reduces VEGF expression in retinal pigment epithelium (RPE) and inhibits RPE-induced sprouting angiogenesis in vitro. *FEBS Lett*. 2008;582(20):3097-102.
62. Ma J, Sun Y, Lopez FJ, Adamson P, Kurali E, Lashkari K. Blockage of PI3K/mTOR Pathways Inhibits Laser-Induced Choroidal Neovascularization and Improves Outcomes Relative to VEGF-A Suppression Alone. *Invest Ophthalmol Vis Sci*. 2016;57(7):3138-44.
63. Kersten E, Paun CC, Schellevis RL, Hoyng CB, Delcourt C, Lengyel I, et al. Systemic and ocular fluid compounds as potential biomarkers in age-related macular degeneration. *Survey of Ophthalmology*. 2018;63(1):9-39.
64. Grassmann F, Schoenberger PGA, Brandl C, Schick T, Hasler D, Meister G, et al. A Circulating MicroRNA Profile Is Associated with Late-Stage Neovascular Age-Related Macular Degeneration. *Plos One*. 2014;9(9).
65. Maturi R, Chhablani J, Harless A, Japeen A, Bracha P. Sirolimus in conjunction with aflibercept vs. aflibercept monotherapy for the treatment of chronic exudative AMD. 35th annual meeting of the American Society of Retina Specialists; Boston, MA2017.
66. Bracha P, Chhablani J, Harless A, Dumpala S, Maturi R. Prospective, randomized, subject-masked evaluation of intravitreal Sirolimus vs. anti-VEGF in

chronic neovascular AMD with persistent retinal fluid: 1-year follow-up. *Investigative Ophthalmology & Visual Science*. 2017;58(8):2967-.

67. Zarbin MA, Rosenfeld PJ. Review of Emerging Treatments for Age-Related Macular Degeneration. In: Stratton RD, Hauswirth WW, Gardner TW, editors. *Studies on Retinal and Choroidal Disorders. Oxidative Stress in Applied Basic Research and Clinical Practice* 2012. p. 1-46.

68. Morgan-Warren PJ, O'Neill J, de Cogan F, Spivak I, Ashush H, Kalinski H, et al. siRNA-Mediated Knockdown of the mTOR Inhibitor RTP801 Promotes Retinal Ganglion Cell Survival and Axon Elongation by Direct and Indirect Mechanisms. *Investigative Ophthalmology & Visual Science*. 2016;57(2):429-43.

69. Nguyen QD, Schachar RA, Nduaka CI, Sperling M, Klamerus KJ, Chi-Burris K, et al. Evaluation of the siRNA PF-04523655 versus Ranibizumab for the Treatment of Neovascular Age-related Macular Degeneration (MONET Study). *Ophthalmology*. 2012;119(9):1867-73.

70. Nguyen QD, Schachar RA, Nduaka CI, Sperling M, Basile AS, Klamerus KJ, et al. Dose-Ranging Evaluation of Intravitreal siRNA PF-04523655 for Diabetic Macular Edema (the DEGAS Study). *Investigative Ophthalmology & Visual Science*. 2012;53(12):7666-74.

71. Thierry M, Pasquis B, Buteau B, Fourgeux C, Dembele D, Leclere L, et al. Early adaptive response of the retina to a pro-diabetogenic diet: Impairment of cone response and gene expression changes in high-fructose fed rats. *Exp Eye Res*. 2015;135:37-46.

72. Wei J, Jiang H, Gao H, Wang G. Blocking Mammalian Target of Rapamycin (mTOR) Attenuates HIF-1alpha Pathways Engaged-Vascular Endothelial Growth Factor (VEGF) in Diabetic Retinopathy. *Cell Physiol Biochem*. 2016;40(6):1570-7.

73. Coughlin BA, Feenstra DJ, Mohr S. Müller cells and diabetic retinopathy. *Vision Research*. 2017;139:93-100.

74. Lopes de Faria JM, Duarte DA, Montemurro C, Papadimitriou A, Consonni SR, Lopes de Faria JB. Defective Autophagy in Diabetic Retinopathy. *Invest Ophthalmol Vis Sci.* 2016;57(10):4356-66.
75. Chung SH, Shen W, Gillies MC. Laser capture microdissection-directed profiling of glycolytic and mTOR pathways in areas of selectively ablated Muller cells in the murine retina. *Invest Ophthalmol Vis Sci.* 2013;54(10):6578-85.
76. Dugel PU, Blumenkranz MS, Haller JA, Williams GA, Solley WA, Kleinman DM, et al. A randomized, dose-escalation study of subconjunctival and intravitreal injections of sirolimus in patients with diabetic macular edema. *Ophthalmology.* 2012;119(1):124-31.
77. Verbakel SK, van Huet RAC, Boon CJF, den Hollander AI, Collin RWJ, Klaver CCW, et al. Non-syndromic retinitis pigmentosa. *Prog Retin Eye Res.* 2018;66:157-86.
78. Campochiaro PA, Mir TA. The mechanism of cone cell death in Retinitis Pigmentosa. *Prog Retin Eye Res.* 2018;62:24-37.
79. Punzo C, Kornacker K, Cepko CL. Stimulation of the insulin/mTOR pathway delays cone death in a mouse model of retinitis pigmentosa. *Nat Neurosci.* 2009;12(1):44-52.
80. Lin B, Xiong G, Yang W. Ribosomal protein S6 kinase 1 promotes the survival of photoreceptors in retinitis pigmentosa. *Cell Death Dis.* 2018;9(12):1141.
81. Venkatesh A, Ma S, Le YZ, Hall MN, Ruegg MA, Punzo C. Activated mTORC1 promotes long-term cone survival in retinitis pigmentosa mice. *J Clin Invest.* 2015;125(4):1446-58.
82. Zhang L, Justus S, Xu Y, Pluchenik T, Hsu CW, Yang J, et al. Reprogramming towards anabolism impedes degeneration in a preclinical model of retinitis pigmentosa. *Hum Mol Genet.* 2016;25(19):4244-55.

83. Venkatesh A, Ma S, Punzo C. TSC but not PTEN loss in starving cones of retinitis pigmentosa mice leads to an autophagy defect and mTORC1 dissociation from the lysosome. *Cell Death Dis.* 2016;7(6):e2279.
84. McDougald DS, Papp TE, Zezulin AU, Zhou S, Bennett J. AKT3 Gene Transfer Promotes Anabolic Reprogramming and Photoreceptor Neuroprotection in a Pre-clinical Model of Retinitis Pigmentosa. *Molecular therapy : the journal of the American Society of Gene Therapy.* 2019;27(7):1313-26.
85. Levkovitch-Verbin H. Animal models of optic nerve diseases. *Eye.* 2004;18(11):1066-74.
86. Morquette B, Morquette P, Agostinone J, Feinstein E, McKinney RA, Kolta A, et al. REDD2-mediated inhibition of mTOR promotes dendrite retraction induced by axonal injury. *Cell Death and Differentiation.* 2015;22(4):612-25.
87. Duan X, Qiao M, Bei F, Kim IJ, He Z, Sanes JR. Subtype-specific regeneration of retinal ganglion cells following axotomy: effects of osteopontin and mTOR signaling. *Neuron.* 2015;85(6):1244-56.
88. Sun F, Park KK, Belin S, Wang D, Lu T, Chen G, et al. Sustained axon regeneration induced by co-deletion of PTEN and SOCS3. *Nature.* 2011;480(7377):372-5.
89. Leibinger M, Andreadaki A, Fischer D. Role of mTOR in neuroprotection and axon regeneration after inflammatory stimulation. *Neurobiol Dis.* 2012;46(2):314-24.
90. Rodriguez-Muela N, Germain F, Marino G, Fitze PS, Boya P. Autophagy promotes survival of retinal ganglion cells after optic nerve axotomy in mice. *Cell Death and Differentiation.* 2012;19(1):162-9.
91. Zhou J, Chen F, Yan A, Xia X. Role of mammalian target of rapamycin in regulating HIF-1alpha and vascular endothelial growth factor signals in glaucoma. *Arch Physiol Biochem.* 2019:1-7.

92. Park HYL, Kim JH, Park CK. Different contributions of autophagy to retinal ganglion cell death in the diabetic and glaucomatous retinas. *Scientific Reports*. 2018;8:9.
93. Kitaoka Y, Munemasa Y, Kojima K, Hirano A, Ueno S, Takagi H. Axonal protection by Nmnat3 overexpression with involvement of autophagy in optic nerve degeneration. *Cell Death & Disease*. 2013;4(10):e860-e.
94. Su WR, Li ZH, Jia Y, Zhuo YH. Rapamycin Is Neuroprotective in a Rat Chronic Hypertensive Glaucoma Model. *Plos One*. 2014;9(6).
95. Koch JC, Lingor P. The role of autophagy in axonal degeneration of the optic nerve. *Experimental Eye Research*. 2016;144:81-9.
96. Porter K, Hirt J, Stamer WD, Liton PB. Autophagic dysregulation in glaucomatous trabecular meshwork cells. *Biochim Biophys Acta-Mol Basis Dis*. 2015;1852(3):379-85.
97. He JN, Zhang SD, Qu Y, Wang HL, Tham CC, Pan CP, et al. Rapamycin Removes Damaged Mitochondria and Protects Human Trabecular Meshwork (TM-1) Cells from Chronic Oxidative Stress. *Mol Neurobiol*. 2019;56(9):6586-93.
98. Lascaratos G, Chau KY, Zhu H, Gkotsi D, Kamal D, Gout I, et al. Systemic PTEN-Akt1-mTOR pathway activity in patients with normal tension glaucoma and ocular hypertension: A case series. *Mitochondrion*. 2017;36:96-102.