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The potential effects of nutrients and light on autophagy-mediated visual function and clearance of retinal aggregates

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ABSTRACT

Increasing findings indicate that a dysfunction in the autophagy machinery is common during retinal degeneration. The present article provides evidence showing that an autophagy defect in the outer retinal layers is commonly described at the onset of retinal degeneration. These findings involve a number of structures placed at the border between the inner choroid and the outer retina encompassing the choriocapillaris, the Bruch's membrane, photoreceptors and Mueller cells. At the center of these anatomical substrates are placed cells forming the retinal pigment epithelium (RPE), where autophagy seems to play most of its effects. In fact, a failure of the autophagy flux is mostly severe at the level of RPE. Among various retinal degenerative disorders, age-related macular degeneration (AMD) is mostly affected by a damage to RPE, which can be reproduced by inhibiting the autophagy machinery and it can be counteracted by the activation of the autophagy pathway. In the present manuscript evidence is provided that such a severe impairment of retinal autophagy may be counteracted by administration of a number of phytochemicals, which possess a strong stimulatory activity on autophagy. Likewise, natural light stimulation administered in the form of pulsatile specific wavelengths is capable of inducing autophagy within the retina. This dual approach to stimulate autophagy is further strengthened by the interaction of light with phytochemicals which is shown to activate the chemical properties of these natural molecules in sustaining retinal integrity. The beneficial effects of photo-biomodulation combined with phytochemicals is based on the removal of toxic lipid, sugar and protein species along with the stimulation of mitochondrial turn-over. Additional effects of autophagy stimulation under the combined effects of nutraceuticals and light pulses are discussed concerning stimulation of retinal stem cells which partly correspond to a subpopulation of RPE cells.

Key words

Age-related macular degeneration • retinal pigment epithelium • light exposure • nutraceuticals • photo-sensitive phytochemicals.

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Introduction

In recent studies we provided evidence that specific nutraceuticals may be effective in removing retinal aggregates and improving retinal integrity in the course of retinal degeneration (Pinelli et al., 2021a). Such an effect is enhanced when nutraceuticals are combined with specific wave-lengths (Pinelli et al., 2022). Since light and phytochemicals share the ability to stimulate the autophagy machinery (Figure 1, Pinelli et al., 2021a), the present manuscript analyses whether such a mechanism may represent a novel tool to improve vision, targeting retinal degeneration and attenuating the production of proteinaceous, sugar and lipid aggregates.

In detail, we specifically focus on the outer retinal segment at the border with the choroid as a potential site where phytochemicals may synergize with specific wave-lengths to empower the autophagy machinery. In fact, between outer retinal and inner choroid membranes polymorphous aggregates known as drusen typically occurs (Figure 2). This mostly happens during a degenerative disorder of the retina, which is known as age-related macular degeneration (AMD). Here, the seminal role of the small choroid blood vessels in the choriocapillaris, the Bruch's membrane (BM) and retinal pigment epithelium (PPE) in regulating the trafficking of proteins lipids and carbohydrates is likely to be involved in the deposition of composite debris visible as drusen, which characterize the pathological culprit of AMD (Figure 2). In addition, in the outer retina, an important role is likely to occur through the metabolic interaction between RPE, outer retinal neurons and outer segment of Muller glial cells (Gabrielle, 2022). All these structures may be crucial to affect drusen deposition and sustain the process of retinal degeneration or provide beneficial effects for retinal integrity and visual processing. In fact, retinal aggregates are often found between the RPE and the choroid (typical drusen), although other aggregates may occur between RPE and retinal neurons (pseudodrusen, Pinelli et al., 2020b; Saßmannshausen et al., 2022; Domalpally et al., 2022; Jeffrey et al., 2022) (Figure 3). This suggests the involvement of both the choroid-RPE junction and the border between RPE, Muller cells (Figure 4) and photoreceptors. Drusen deposition

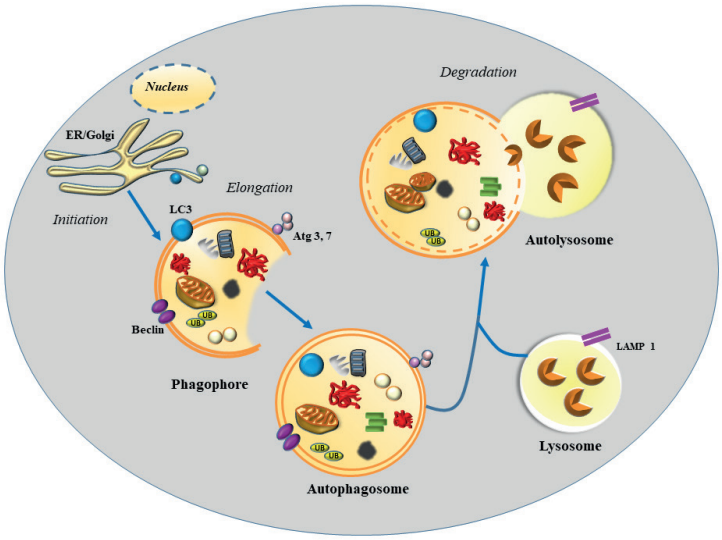

Fig. 1 - Schematic view of the autophagy pathway. The autophagy pathway has a major role in removing a variety of lipid, sugar and protein substrates which may be altered during physiological metabolism. The role of autophagy extends to the removal of mitochondria. The nascent autophagy structure is named phagophore which is produced from the trans-Gogi network upon stimulation of specific molecules such as Beclin, LC3, Atg 3 Atg 7. The elongation of the phagophore and the ceiling of its membrane lead to a vacuole named autophagosome, which is stained for LC3 and Beclin as classic markers. The merging of the autophagosome with lysosome stained by the marker LAMP 1 leads to the digestion of multiple structures. Thus, the classic role of the autophagy machinery consists in removing a variety of substrates through their entrapment within autophagosomes. In this way, the essential definition of autophagy is to provide the removal of altered proteins, lipids, sugars and damaged organelles.

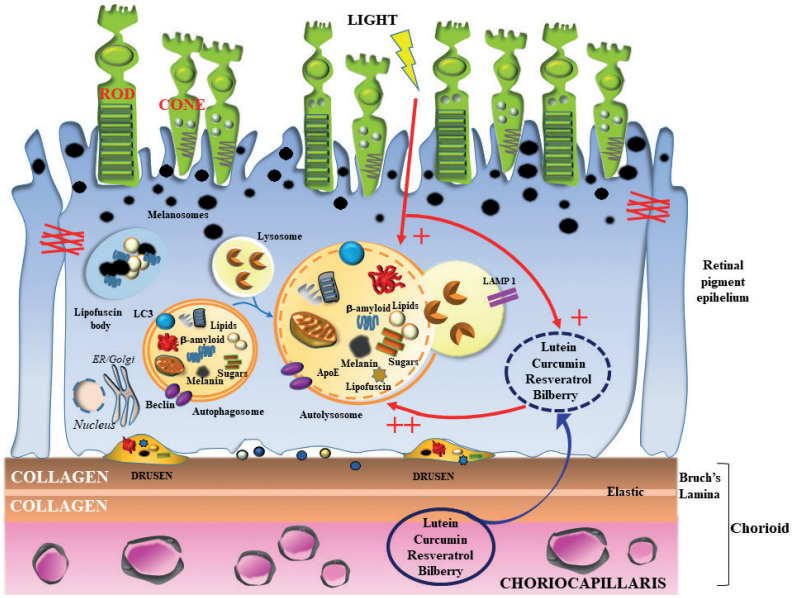

Fig. 2 - Autophagy in the retinal pigment epithelium. The very same classes of molecules and organelles which are removed by autophagy as generally reported in Figure 1 are present in the retinal pigment epithelium (RPE), where specific compounds are more represented. For instance cell debris from photoreceptors, visual proteins such as opsins, melanosomes, and lipofuscin along with amyloid structures are present at this level. When systemic phytochemicals (lutein, curcumin, resveratrol, bilberry) are administered they are able to stimulate the autophagy pathway to form autophagosomes and accelerate their merging with lysosomes to speed the autophagy flux. Similarly, specific wavelengths when applied in pulses sort a similar stimulation of the autophagy machinery. In addition, light may activate phytochemicals by altering their chemical reactivity to improve their autophagy-stimulating effects. Some phytochemical such as lutein are densely accumulated in the RPE following systemic administration with a high concentration within the macula lutea.

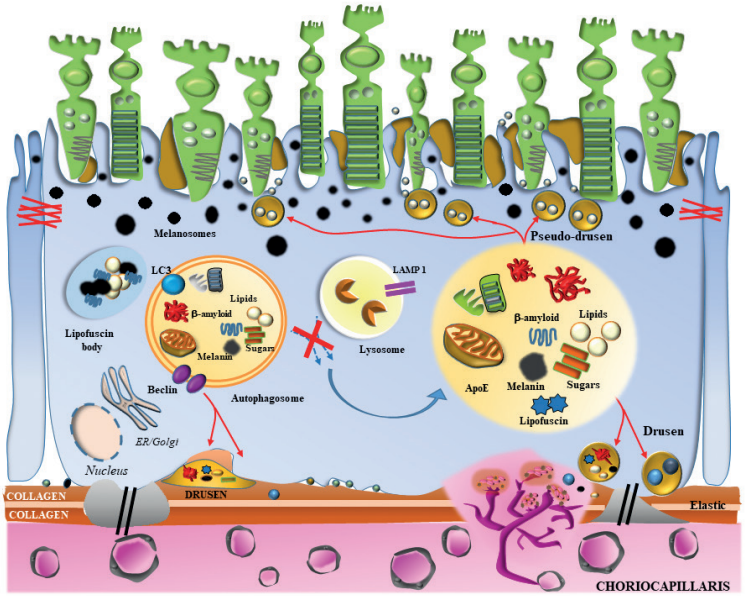

Fig. 3 - Autophagy failure induces a defect in the retinal pigment epithelium (RPE). When autophagy is impaired either due to an excess of substrates or as a consequence of biochemical defect in the RPE autophagosomes do not work properly, leaving intracellular aggregates containing autophagy substrates. Similarly a defect in the merging between autophagosomes and lysosomes leads to stagnant autophagosomes within RPE cells. These stagnant cargoes may damage the RPE cells or being massively released the lead to the accumulation of drusen between the RPE and Bruch's membrane. Similarly the cycling of these aggregates towards the photoreceptors may lead to drusenoid. This toxic cargo may induce neo-angiogenesis and inflammation, which characterizes the wet phenotype of AMD.

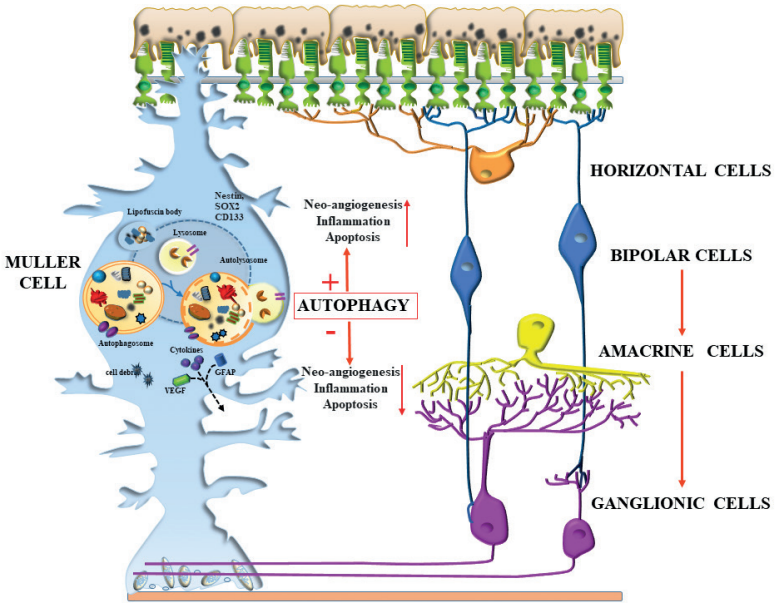

Fig. 4 - Autophagy within Muller cells. In the outer retina, an important role is likely to occur through the metabolic interaction between RPE, outer retinal neurons and outer segment of Muller glial cells. Even these cells feature an active autophagy activity which is relevant to regulate trans-synaptically retinal homeostasis. In detail, a defective autophagy within Muller cells participate to the inflammatory response and the formation of blood vessels. In fact, autophagy activation within Muller cells occludes both neo-angiogenesis and inflammation, which characterize retinal degeneration. Again, autophagy activation rescues apoptosis of Muller cells, which occurs in the course of autophagy defect during retinal degeneration.

occurs most frequently in the extracellular space, which lies between RPE cells and the Bruch's membrane (Figure 2) where extracellular debris owing various volume and shape occur (Jaeger et al., 2008; Pinelli et al., 2020b). To a lesser extent, even the opposite domain of RPE cells, which faces photo-receptors may participate in the deposition of extracellular aggregates known as pseudo-drusen (Saßmannshausen et al., 2022; Domalpally et al., 2022; Jeffrey et al., 2022). Since the autophagy activity in these structures appears to be crucial in the process of removing metabolic substrates, the working hypothesis of the present contribution postulates that a defect in the autophagy pathway within these cells may trigger neurodegeneration, along with aggregates deposition, and ultimately fostering the maturation of these aggregates in the course of retinal degeneration (Figure 3). The role of photo-modulation combined with specific nutraceuticals as a novel approach to prevent and/or remove these aggregates via activation of the autophagy machinery is discussed (Figure 2). The classic role, which is attributed to the autophagy machinery consists in removing a variety of substrates through their entrapment within specific organelles named autophagosomes (Figure 1). In this way, the essential definition of autophagy is to provide the removal of altered proteins, lipids, sugars and damaged organelles. These molecules and organelles in turn are aggregated within stagnant lysosomes within retinal cells, when a fast or slow retinal degenerative disorders takes place (Blasiak et al. 2014, Nita et al. 2020, Bilbao-Malavé et al. 2021, Yako et al. 2021). In this way, autophagy in the retina as well as in other areas of the CNS is seminal to prevent neurodegeneration. In fact, in the retina, just like within a number of areas involved in CNS disorders, an altered autophagy is seminal to promote chronic degenerative conditions (Ravikumar and Rubinsztein, 2004; Rubinsztein et al., 2005; Fornai et al., 2008a, 2008b; Castino et al., 2008; Ferrucci et al., 2008; Isidoro et al., 2009; Madeo et al., 2009).

The prevalence of AMD

Age-related macular degeneration (AMD) is a disorder which includes many diseases owing various etiologies, this accounts for 8.7% of all cases of blindness and its global prevalence is estimated

to increase: 196 million people by year 2020, and 288 million by 2040 (Klein et al., 2004; Wong et al., 2014). As reported by Hyttinen et al. (2017) AMD represents a complex degenerative and progressive disease, which may be induced by abnormal dietary regime, smoking, oxidative stress, cardiovascular disorders and other systemic deleterious factors (Black and Clark, 2015; Fritsche et al., 2014; Kaarniranta et al., 2013). These disorders are characterized by a widespread degeneration, which selectively involves the macular region of the retina producing altered vision with wavy lines, loss of contrast sensitivity and visual acuity. The visual impairment eventually progresses up to irreversible blindness. The prevalence of AMD is very high and it is increasing dramatically in the last decades. In Western countries it represents the main disorder leading to irreversible loss of central vision and blindness. In fact, AMD produces roughly 10% of all cases of blindness and its prevalence is estimated to reach approximately 300 millions of patients by 2040 (Klein et al., 2004; Wong et al., 2014). The prevalence of the disease in the first decade of the new millennium was estimated over 8 million in the United Sates, affecting 4% of the population over the age of 60 years (de Jong, 2006). However, it is predicted that such a prevalence at present is dramatically increasing, mostly among Western Countries, where it increased several-fold (Datta et al., 2017). This mostly concerns the incidence of the atrophic variant of AMD. This atrophic variant, which is also known as "dry" variant involves most (80%) of people diagnosed with AMD. This variant is defined by a specific pathology, time course, and disease severity. This is distinct from the other variant known as "wet" AMD which is characterized by exudates from retinal vessels with neo-angiogenesis fast progression and a poor prognosis (Pascolini et al., 2004; Congdon et al., 2004; Jager et al., 2008; Pinelli et al., 2020b). Although classic nosography distinguishes between two AMD phenotypes, the dry form may slowly progress into the wet one, which otherwise may be already "wet at the onset". In these cases, the wet AMD emerges abruptly and rapidly progresses into blindness. Thus, blindness is the final outcome of both forms, although the dry variant progresses at a slower rate. This is why AMD is one of the leading causes of irreversible loss of central vision and blindness worldwide, especially in the developed countries. This is dramatic also considering that effective treatments at present are limited. In fact, AMD represents a complex degenerative progressive disorder. While age is the primary risk factor for AMD, dietary regime, smoking, oxidative stress, and cardiovascular are also contributing factors, along with genetic susceptibility (at least 20 genetic risk loci known so far, Black and Clark, 2015; Fritsche et al., 2014; Kaarniranta et al., 2013).

The onset and maturation of AMD

The complex scenario where AMD may appear includes a variety of conditions, which were listed above including age, dietary regime, smoking, oxidative stress, cardiovascular malfunction and genetic susceptibility (Black and Clark, 2015; Fritsche et al., 2014; Kaarniranta et al., 2013; Hyttinen et al., 2017). These various potential causes of AMD would determine different disorders. Nonetheless, elementary pathology and cellular as well as extra-cellular alterations are similar, which suggests that various risk factors and different etiologies converge to alter a final common pathway to initiate and sustain disease progression. In a recent manuscript by Hyttinen et al. (2017), autophagy acting on the RPE cells is claimed to play a substantial role in the course of AMD.

In fact, although a number of biochemical pathways contribute to sustain retinal integrity, the autophagy machinery plays a leading role in preserving cell viability and designing retinal anatomy.

Since it is generally assumed that two disease phenotypes can be distinguished, some considerations are needed to describe the onset of the disorder, which differ concerning pathology, time course, and severity. These two classic forms, were above defined as "dry" and "wet" AMD (Figure 3). However, such a distinction remains quite rough since, a number of overlapping conditions occur as a continuum between these two phenotypes (Pinelli et al., 2020b; Ambati and Fowler, 2012). In an effort to be adherent as much as possible to disease variability and its pathological *continuum*, we may consider that, at onset the prevalent form is dry AMD (85%). However, a number of wet AMD derives from the maturation of the dry form. This implies extreme care in formulating a clear-cut distinction when

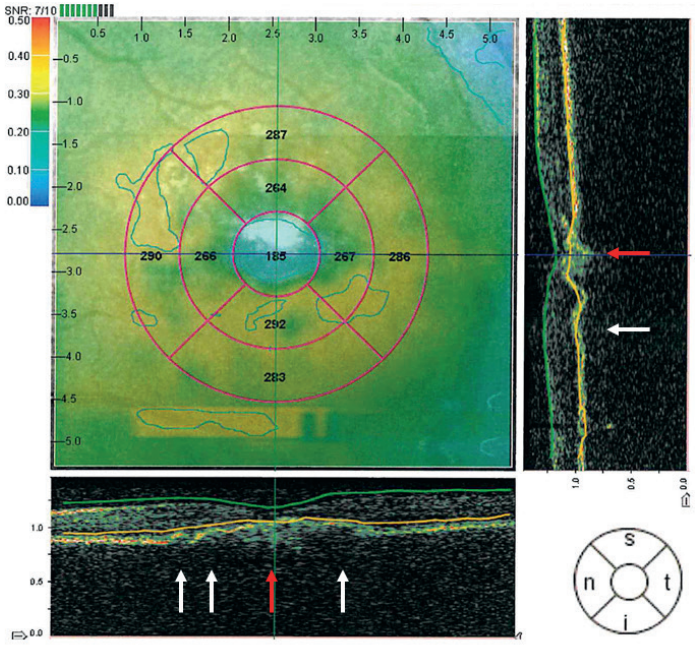

Fig. 5 - OCT-based retinal topography with map diagram after PBM treatment, at 6 months. Retinal topography based on OCT detected in the horizontal and vertical axis shows a disruption of the retinal planar arrangement induced by the drusen (red and white arrows), the loss of thickness of the retinal epithelium is mostly evident in the foveal region (from Pinelli et al., 2021a).

defining AMD pathology. We may say that, when AMD is observed in its dry stage the progression of retinal degeneration is slower (Seddon, 2001) and it produces a slighter loss of visual acuity (Jager et al., 2008). The progression of dry AMD eventually leads to blindness although this may be the consequence of a shift from dry into wet disease phenotypes. Apart from being a disease continuum both classic wet and dry phenotypes share the pathological hallmark of extra-cellular aggregates nearby the RPE named drusen. Drusen develop from the aggregation of various components including lipids, sugars and proteins, where beta amyloid is considered to be a classic hallmark (Figure 3). Actually, drusen appear as polymorphous debris at neuropathology exam and when they are observed at OCT they are detected a dense bodies (Figure 5), which enlarge the space between the retinal pigment epithelium and Bruch's membrane or may develop as pseudo-drusen enlarging the tight connection between the RPE and photoreceptors (Pinelli et al., 2020b). The classic concept which binds neuropathology and symptoms in AMD assesses that drusen *per se* would worsen visual acuity. In the course of macular degeneration, at early stages the RPE appears to be thinned and irregular, while Bruch's membrane is thickened (Hyttinen et al., 2017). At these stages the deposition of drusen increases dramatically over time to lead to a massive damage of photoreceptors (Hyttinen et al., 2017). This is believed to be the mostly the consequence of mechanical alterations, which are produced by these polymorphic aggregates, which are supposed to alter retinal planarity and shape of photoreceptor, thus impairing the homogeneity of the retinal layer where the light quanta impact photoreceptors to impede their correct function. In fact, it is well accepted that, amount and area of drusen directly relate to the disease state, since their amount and size and site specificity within the macula all determine the severity of AMD. Indeed, recently this mechanical concept was quite challenged by data showing that, the specific measurement of the drusenoid area in the macula does not indicate necessarily a drusen-dependent damage (Pinelli et al., 2020a). In fact, despite a significant difference in the number

of drusen exists when comparing patients with mild and severe AMD-induced visual impairment, a significant correlation between the extent of the drusenoid area in the macula and the severity of visual impairment does not occur. This suggests that drusen and visual deterioration both occur in AMD without establishing a causal link. Thus, loss of vision may not be a direct mechanical influence of drusen on photoreceptors but rather a concomitant effects due to biochemical defects. These defects would lead from one side to drusen development, while it may also lead to visual deterioration without a strict correlation between each other. According to this hypothesis both phenomena would develop as a consequence of an upstream biochemical alterations without implying that drusen do produce visual deterioration (Pinelli et al., 2020a, 2020b). For instance, we reported specific clinical conditions where visual impairment is very severe with only a slight alteration in the planar pattern of the retina or, vice versa, an extended drusenoid area occurs while fair visual acuity and contrast sensitivity is preserved, in the absence of of metamorphopsia (Pinelli et al., 2020a). These discrepancies occur keeping constant the macular region under analysis. A biochemical analysis of key areas and specific domains at retinal-choroid border should help to better disclose the real morpho-functional deficit, which takes place in AMD and whether this causes drusen or visual impairment or both (Pinelli et al., 2020b).

In the wet variant of AMD drusen occur along with development of newly formed blood vessels (Figure 3) in the retina beyond the choroid-retinal border. These newly formed vessels are abundant, dense and frail, thus causing bleeding and altered wall permeability, which leads to fluids accumulation in the extracellular space. This explains the definition of such a disease phenotype of AMD as the "wet" variant. New vessels and fluid accumulation are absent in the dry variant. In both cases, the anatomical sites, which takes a center stage in both variants of AMD is the inner choroid and outer retina. In fact, the retina is mostly involved concerning its external domain, which faces the Bruch's membrane and blood vessels of the choroid and partly its internal domain, which embraces photoreceptors and the limiting membrane of Muller cells. The dry variant is typically featured by plain drusen, which are placed between RPE and the Bruch's membrane without extending to blood vessels of the choroid. In contrast, in the wet variant the Bruch's membrane loses integrity and it does no longer produce a barrier between the blood vessels and RPE (Figure 3). Rather, small vessels extend from the choroid to the retina. These vessels surpass the retina-choroid border through the ruptures of the Bruch's membrane, thus the separation border between the choroid and the retina is destroyed. In this way, the outer retinal segment, which in normal conditions does not contain blood vessels, now develops abnormal vessels, which leads to bleeding and exudates.

Although these specific phenomena are emphasized concerning the foveal region, this may occur all over the retina and this is often the case of advanced stages of AMD, where a geographic alteration encompasses the whole retina. However, the typical site, which requires to be affected to configure a classic AMD by definition remains the macular region. This lies in the posterior retina, placed almost in center on the temporal side. This retinal region is thin and it possesses tiny cones, which in the absence of blood vessels and inner retinal neurons are directly impacted by light to allow the best visual acuity, contrast sensitivity and chromatic discrimination. By definition, AMD in order to be diagnosed needs to affects this area, at early stages being visible as drusen or pseudodrusen in the choroid-retinal border. Due to the direct impact of light on the outer retina in this region it is not surprising that the effects of light stimulation may impact degeneration of the foveal region of the retina

The physiological role of the retinal pigment epithelium (Figure 2)

The retinal pigment epithelium (RPE) derives from the outer membrane of an embryonic protrusion from the diencephalon, which produces the neural membrane of the eyeball. The RPE is a mono-layer of melanin-containing neural cells which produce melanin upon the stimulation of tyrosine hydroxylase (Roffler-Tarlov et al., 2013; Pinelli et al., 2021b) and embrace the photo-receptors with their distal membrane convolution. The melanin bodies, which are present in the distal part of these cells impede the spreading of light quanta from

the photoreceptor which is mostly stimulated by light to the closest one in order to keep spatial discrimination during visual stimulation. The role or RPE in the retina is fundamental to modulate its physiology and preserving retinal integrity. In fact, it is believed that RPE may impede neurodegeneration and occlude neuroinflammation. The presence of melanin within RPE cells is a powerful regulator of the cell shape and phenotype. In fact, as described in Pinelli et al., 2021b when comparing albino and pigmented retinas, the presence of melanin alters the cell structure. In fact, light microscopy indicates that, in the absence of melanin the cells forming the RPE appear flat and lack cell protrusions. In detail, the albino RPE does not intermingle with its cell processes between cones and rodes of the outer retina (Pinelli et al., 2021b). Thus, the presence of melanin in the RPE is associated with cell thickness and formation of cell processes which separate close photoreceptors to impede light to transmit from cell to cell through lateral domains. The thickness of pigmented RPE is partly explained by the presence of melanin bodies, which fill the cells just like skin melanocytes. These melanin bodies push the pigment towards cell processes to fill the space between the outer segments of photoreceptors. The occurrence of melanin within the RPE strongly depends on the integrity of TH-containing fibers forming the sympathetic innervation to the retina. In fact, in albino retinas these fibers are lacking (Pinelli et al., 2021b). In this way, it may be inferred that sympathetic innervation of RPE modulates cell volume and phenotype including cell biochemistry. Occurrence of melanin within RPE, apart from granting a selective stimulation of those photoreceptors directly impacted by light, also avoids an excess of light stimulation, which may produce toxic reactive oxygen species and free radicals. This protects from short wave-length light and chemical toxic species, since the chemical properties of melanin allows this molecule to buffer protein misfolding which naturally occurs and may trigger retinal degeneration. These effects of melanin are supposed to modulate the protein to form aggregates in the retina, which is also induced by the ability of melanin to bind to lipid and sugar structures occurring within intra-and extra-cellular inclusions. This is expected to alter the amount of drusen or other retinal aggregates. The occurrence of melanin despite fostering extracellular aggregates is expected to protect the retina from toxic species. In line with this, these Authors could not detect drusen from the degenerating retina occurring in albino patients. In these cases retinal degeneration takes place in the absence of extracellular aggregates and the presence of a flat retinal planar arrangement. This further witnesses for a divergence between drusen and visual deterioration since these phenomena may be merely associated. In fact, although melanin fosters aggregates it protects from retinal degeneration. Such a fascinating evidence may be explained by the inert nature or even protective role of extracellular aggregates in the course of retinal degeneration. In fact, Herrera et al. (2020) indicate that melanin is beneficial to counteract cell damage in the course of retinal degeneration. This poses the questions back on whether inclusions during neurodegenerative disorders may be beneficial rather than contributing to cell damage (Fornai et al., 2005; Lazzeri et al., 2007). In fact, the ability to entrap toxic species within inclusions may prevent their detrimental effects and impede the spreading of degeneration to neighboring cells.

A critical role of RPE is related to its trophic effects since it provides nutrients from the inner choroid to the outer segment of photoreceptors. Back again, the recycling of the outer segment and opsins from photoreceptors is a key function of the RPE along with its synthetic activity. All these effects strongly rely on effective metabolic activity within RPE cells, which is mostly centered on the autophagy machinery.

The autophagy in the retina

Hyttinen et al. (2017) clearly indicated the peculiarity of the retinal tissue in relationship with oxygen consumption. In fact, as a real part of the central nervous system the retina is composed of neural cells, which produce a high oxygen extraction. This is amplified by the specific role of the retina as a recipient of light stimulation and its high content in light-sensitive molecular species. This dramatically increases the rate of oxygen consumption and produces an environment, which is susceptible to damage produced by highly reactive oxygen species. In these physiological conditions the damage to specific chemical species including

proteins and lipids is very frequent and abnormal metabolic products are likely to occur. These phenomena underlie the multiple etiology of retinal degeneration. These include: oxidative injury, DNA damage and chronic inflammation (Bales and Gross, 2016; Kaarniranta et al., 2013; Querques et al., 2014; Jarrett and Boulton, 2012). Accumulation of altered sugars, lipids and proteins require powerful systems to degrade and clear these compounds from the retina. In this way, proteinaceous material and lipofuscin prone to aggregate may be eliminated, thus preventing their accumulation into drusen. In fact, in the presence of altered lipid aggregates protein misfolding is increased, which leads to the enhancement of drusen formation (Terman and Brunk, 2004; Kaemmerer et al., 2007; Kaarniranta et al., 2009). This requires the presence of metabolic systems, which may be effective in counteracting the amount of toxic compounds. Several pathways contribute to these protective effects. Among these, protein and lipid clearance provided by autophagy is relevant Kaarniranta et al., 2013; Hyttinen et al., 2017; Pinelli et al., 2020b). In fact, it is well known that autophagy defects lead to accumulation of misfolded proteins and organelles (Hyttinen et al., 2013, 2014; Kaarniranta et al., 2013; Kaarniranta et al., 2017). The loss of autophagy progression leads to the storage of misfolded proteins within lysosomes, where they can be found as advanced glycation end products (AGEs) along with lipid material in the form of lipofuscin. These molecules may be found already as cellular debris within stagnant lysosomes to represent precursors of drusen, which may eventually be released from the cells (Luzio et al., 2007). Among cell debris a number of damaged organelles can be found including the endoplasmic reticulum and mitochondria. The lack of an effective autophagy clearance leads to accumulate also cell debris, which derive from the phagocytosis of the outer photoreceptor segment (Mitter et al., 2014).

As reported by Hyttinen et al (2017) there is a noticeable overlapping among undigested material which makes lipofuscin and melanosomes very heterogeneous mostly upon autophagy defect. Thus, lipofuscin may feature a mixture of lipid, sugars, and proteins along with photopigment residues and altered cell organelles. These lipofuscines can stay in the cells persistently within stagnant lysosomes (Boyer et al., 2012; Kaarniranta et al., 2010; Terman and Brunk, 2004). The autophagy defect, which sorts its effects on a variety of molecules and organelles is considered not to be dangerous at early stages but when the volume becomes considerable. Still the volume effect is likely to play a secondary role in impairing vision compared with the biochemical defect which derives from a defective autophagy. It is remarkable that most of the studies on the role of autophagy in retinal degeneration focused on RPE. In fact, a similar role is likely to be relevant for Muller cells where autophagy is supposed to regulate trans-synaptically retinal homeostasis. This appears to be relevant for the inflammatory response and the formation of blood vessels, which is induced by Muller cells (MC). In fact, autophagy activation within MC occludes both neo-angiogenesis and inflammation, which characterize retinal degeneration (Subirada et al., 2022). Again, autophagy activation rescues apoptosis of Muller cells, which occurs in the course of retinal degeneration (Wang et al., 2019a).

The regulation of autophagy in the inner choroid remains largely non-explored and deserves further investigations. In fact, autophagy regulates the thickening of the Bruch's membrane (Vessey et al., 2022). In the retinal degeneration the dysfunction of the Bruch's membrane appears to be closely related to an autophagy-dependent alteration at the level of pericytes and endothelial cells in the choriocapillaris (Nag et al., 2021). In fact, a rare disorder of the choriocapillaris, with increased angiogenesis and bleeding produces macular drusen deposition (Choi et al., 2022). The role of autophagy in photoreceptors is worth to be investigated as well. In fact, Intartaglia et al (2022) recently found that autophagy prevents cell death of retinal photoreceptors (Intartaglia et al., 2022) in the course of retinal degeneration. The role of autophagy in retinal degeneration is increasingly recognized. This mostly focuses on the transition zone between the epithelial/mesenchymal part of the eye where the choroid-retinal junction occurs (Pinelli et al., 2020b; Kaarniranta et al., 2022). At this level multiple cell types and organelles and molecules are likely to be affected, which can be modulated via autophagy induction. This calls for designing novel therapies to treat retinal degeneration. An emphasis should be posed on macular degeneration where the process starts right at the interface between choroid and retina.

Altered autophagy may impair the visual process independently from AMD neuropathology

In the hypothesis that occurrence of drusen is not directly responsible for visual symptoms in AMD and it rather accompanies the loss of visual acuity due to a common upstream biochemical dysfunction, one should hypothesize that visual symptoms and drusenoid area are not necessarily related. Indeed, evidence for such a dissociation were published a few months ago Pinelli et al., (2020a). In line with this, the very same biochemical alterations, which are responsible for drusen formation and loss of planar retinal arrangement, should be responsible at molecular level for the loss of visual acuity. A recent manuscript by Intartaglia et al. (2021) confirms the need of an effective autophagy in the choroid-retinal border. This is bound to the multiple functions exerted at this level, mostly by cells of the RPE which are in need to specific spatial and temporal gene regulation to cope with digestion and recycling of intracellular and photoreceptor-derived components in response to daily light and stress conditions. This is why the autophagy machinery in this part of the retina needs to be finely tuned both concerning transcription of specific genes and protein expression to maintain and treating visual impairments in patients affected by retinal disorders (Intartaglia et al., 2021). In fact, in keeping with the autophagy hypothesis a recent manuscript indicate that autophagy impairment stimulation improves visual acuity due to a direct effect on retinal metabolism (Kim et al., 2021), independently by concomitant or absent retinal neuropathology. These authors demonstrate that, the RPE produces a molecule named retinal pigment epithelium derived factor (RPEDF) which restores the visual cycle. The role of autophagy in the process of vision is further substantiated by Datta et al., (2022). In fact, considering the high rate of daily phagocytosis of the outer segment of photoreceptor, unique photo-oxidative stress, and high metabolism for maintaining vision, the effective role of autophagy within the RPE is needed to provide vision. This is why the RPE has robust macro-autophagy/autophagy, and mitochondrial and antioxidant networks. This is reciprocated by Intartaglia et al. (2022) who show how, an intact autophagy is needed for providing appropriate visual function, while impairment of autophagy is key in the visual loss in retinitis pigmentosa and inherited retinal dystrophies. In detail, the inhibition of the Ezrin, which is an endogenous inhibitor of the autophagy pathway, protects from the cell death of photoreceptors and preserves visual ability in a model of retinitis pigmentosa (Intartaglia et al., 2022). Based on their findings these authors postulate that pulsatile induction of autophagy may improve therapeutic strategies to treat devastating retinal disorders (Intartaglia et al., 2022). The needs of an effective autophagy in the process of vision is in line with the stimulating effects of light on autophagy structures. In fact, autophagy is diurnally regulated in normal rods, with more autophagic structures generated during periods of light, and this regulation is non-circadian but directly induced by light exposure (Wen et al., 2019). In fact, autophagy is induced by the process of photo-transduction. Also in diabetic retinopathy, the loss of visual acuity is associated with an altered expression of a number (23) autophagy-related genes (Wang et al., 2022). As reported by Santo and Conte (2021), an effective progression of autophagy to lysosomal compartment is critical to maintain vision. In fact, lysosomal inhibition is deleterious for photoreceptor homeostasis and produces a negative impact on the process of vision. These concepts prompted the authors to plan therapeutic strategies aimed to improve lysosomal activity as a treatment for blindness. Most of these studies are focusing on correcting the impairment of lysosome/autophagy system as a strategy to improve visual homeostasis (Santo and Conte, 2021).

The role of nutraceuticals

Classic drug currently used to treat macular degeneration do not provide at present a solution to the disorder and do not significantly alter disease progression. The strong limit of designing a disease-modifying therapy in AMD is burdened by the long disease course and the critical side effects that most drug induce when administered at high doses or for prolonged time intervals. The pathology and patho-biochemistry of AMD and most retinal degenerations indicate a marked defect in the autophagy machinery, which acts at multiple levels in the visual detection and it is responsible for most

pathological alterations. This suggests a natural remedy to modulate retinal integrity by exploiting those pathways which exists in the retina, which are bound to the process of photo-detection. From one side this immediately recalls the natural role of light in stimulating retinal function. From another side photo-sensitive pigments, which are stimulated by light may represent a natural background to preserve and/or rejuvenate a degenerating retina. In fact, when considering that an autophagy defect is likely to occur in AMD it is surprising that a number of phytochemical and other autophagy-stimulating nutraceuticals do possess a photosensitive moiety, which is impacted by light exposure. Specific phytochemicals and other natural nutraceuticals are known to be powerful stimulators of the autophagy machinery and can be administered for long time intervals, in the absence of noticeable side-effects. This is the case of lutein, curcumin, resveratrol Vaccinium Myrtillus and many other compounds, which at present are intensely investigated. Lutein possesses a high concentration in the retina where it naturally gets stored in the macular region (which in fact is defined *macula lutea*). Such a pigment stimulates visual acuity and color detection right in the site where it naturally accumulates. In fact, the macular region is rich in lutein along with its isomer zeaxanthin and its metabolite meso-zeaxanthin (Algan et al., 2022). Since human beings cannot synthesize lutein, zeaxanthin, and meso-zeaxanthin, these three xanthophyll carotenoid pigments can be administered in the diet. In fact, following a diet supplementation these compounds selectively concentrate in the center of the retina (Li et al., 2022a, b). The natural source of these luteins in the diet comes from orange-and yellow-colored vegetables, yellow corn, and egg yolks, Goji berries contain the highest levels of zeaxanthin of any food. It is remarkable how recent studies indicate that lutein stimulates autophagy and it is effective in preserving autophagy in the presence of autophagy inhibition (Munia et al., 2020).

In detail, lutein was tested as an autophagy inducer by Chang et al., (2017). These authors found that lutein, apart from its anti-oxidant effects can also induce autophagy for cell survival. In fact, lutein dose-dependently induces accumulation of LC3-positive puncta and it stimulates autophagosomes formation. In addition, lutein upregulates some autophagy-related genes including ATG4A, ATG5, ATG7, ATG12, and beclin-1 (BENC1) and increases the expression of the protein beclin1. This latter effect appear to be key in the autophagy stimulating properties of lutein since in the absence of beclin1 (BECN1 knockdown models) autophagy is no longer stimulated by lutein (Chang et al., 2017).

Among nutraceuticals resveratrol is a powerful autophagy inducer, in fact following resveratrol Munia et al (2020) described autophagy activation in the retina at the level of the RPE. Within these cells resveratrol increases autophagy vacuoles, it augments the LC3II/I ratio while decreasing p62 expression. Even in the case of resveratrol, the effects on the autophagy machinery were independent of mTOR modulation, since autophagy was stimulated downstream of mTOR and the upstream inhibitor of autophagy, 3-methyladenine could not inhibit resveratrol-induced autophagy in RPE cells. The pro-autophagy effects of resveratrol are associated to anti-inflammatory effects in ARPE-19 cells (a cell model of RPE, Josifovska et al., 2020). As previously reported, macular degeneration is characterized by disrupted mitochondria which are present within drusen, lipofuscines and melanosomes. In fact, autophagy is key in regulating the quality control for mitochondria in the cells. This is why upon autophagy induction there is marked stimulation of mitophagy and mitochondrial biogenesis. In the presence of resveratrol mitochondrial function improves. In detail, resveratrol was shown to increase mitochondrial quality and function in zebrafish retinas (Wang et al., 2019b).

Vaccinium Myrtillus, which is also named bilberry belong to blueberry polyphenols family. This compound promotes autophagy and clears misfolded proteins (Li et al., 2022b) as shown for beta-amyloid aggregates (Vepsäläinen et al., 2013). Blueberry extracts promote autophagy and clear lipd deposition (Zhughe et al., 2020). These effects seem to be mediated by a specific metabolite, Protocatechuic acid which promotes the autophagy flux (Li et al., 2022b).

A very promising approach to promote autophagy through naturally occurring compounds is represented by the phytochemical curcumin. In fact curcumin is a powerful autophagy inducer in a variety of cell types in baseline and pathological conditions (Aoki et al., 2007; Shinojima et al., 2007;

Linanaqi et al., 2019; Ryskalin et al., 2020, 2021; Dlamini et al., 2022; Jin et al., 2022; Teng et al., 2022). Specifically, within the retina it is remarkable that curcuma may counteract neurodegeneration as recently indicated by Hassanzadeh er al. (2022), who found that curcuma extract (Cur) exerted a neuroprotective effect in the course of retinal degeneration. Importantly, curcumin modulates apoptosis and MAPK signaling pathway activation thus preventing cell death. A number of studies were reviewed by Chandrasekaran and Madanagopalan (2022) on the protective effects of curcumin in retinal disorders. Most specifically, recent evidence indicate that curcumin possess significant protective effects in the course of age related macular degeneration (Allegrini et al., 2021, 2022; Vallée, 2022).

In line with the evidence that specific nutraceuticals may exert neuroprotective effects on macular degeneration, we recently described a case report, where prolonged administration of specific nutraceuticals appear to be beneficial in the course of dry age-related macular degeneration (AMD). In this study, the patient was administered lutein, resveratrol and Vaccinium Myrtillus all known to be powerful autophagy inducers and effective in modulating autophagy at retinal level. When these compounds were supplemented an improvement of visual acuity and a long-lasting decrease in drusen volume and number was documented. The concomitant intake of lutein, resveratrol and Vaccinium Myrtillus was carried out for six months and it produced a marked decrease in the number and surface of drusen observed at OCT at the 6-month follow-up (Pinelli et al., 2020a, 2020b). At this time interval, the patient experienced a noticeable improvement in visual acuity, a decrease in eye strain, more color contrast, higher visual definition. The case report indicates the potential benefit of nutraceuticals treatment with improved quality of vision in dry AMD. This study is now replicated in a larger population followed over a long-term period. The support of nutraceuticals could therefore offer a new non-invasive, adverse-effect free, which may restore the pathology affecting the cross talk between choroid and retinal cells.

Natural light stimulation

The term photobiomodulation (PBM) refers to the natural light stimulation of biological appropriate targets. According to recent works (Pinelli et al., 2021a) photobiomodulation consists of pulses of near-infrared (NIR) light (500–1000 nm) produced by a laser or non-coherent light sources such as light emitting diodes (LEDs). Light in this wavelength range stimulates a number of cellular function via activation of photo-acceptors (Rojas et al. 2008; Tata and Waynant 2010; Rojas and Gonzalaz-Lima 2011). The use of PBM, when applied according to specific pulses of various time intervals and wave-lenghts is named the “Lugano protocol”. This protocol includes wide wavelengths including amber light to near infra-red light. It is fascinating that the effects of these wavelengths converge on the cellular targets which are common to nutraceuticals. In fact, it is remarkable, though not surprising, that autophagy is promoted both by these specific wave-lengths as well as the nutraceutical discussed above. For instance, amber light (590 nm) activatse the autophagy machinery by acting at multiple levels, modulating a number of autophagy steps and increasing specifically autophagy-committed molecules. Among these, amber light promotes the shifting from the non-active form of the autophagy promoter LC3 I into its active lipidated structure, which represents the active protein LC3 II. Similarly, stimulation of the retina with amber light increases the expression of an upstream autophagy inducer known as Atg5. This occurs both by increasing the primary gene transcript and its protein. Amber light enhances the autophagy flux by activating lysosomal degradation. In fact, exposure to amber light removes the inhibition of lysosomal activity exerted by leupeptin/ NH4Cl. In this way, exposure to amber light is able to clear critical autophagy substrates, which accumulate in the course of retinal degeneration such as lipid droplets (Choi et al., 2016). In keeping with the continuum of low wave-length stimulation, the exposure to pure red light exerts a powerful effect in clearing the autophagy-dependent protein tau most concerning its misfolded conformation. Red light stimulation counteracts the deleterious effects of toxic species and enhances the autophagy machinery also by increasing the chaperonine HSP70 in its inducible isoform. Consistently, as shown for nutraceuticals and amber

light, also red light exposure promotes the expression of the net amount of the autophagy protein LC3. In line with this, the other classic autophagy marker Beclin-1 is increased following red light exposure (Stefenon et al. 2021). Similarly to amber light, red light promotes a dramatic increase in the levels of mRNA coding for the autophagy inducer Atg5. It is not surprising that the overall increase induced by red and amber light in autophagy-related molecules is associated at sub-cellular level with an increase in autophagy related organelles. In fact, red light augments the amount of autophagosomes (Yang et al., 2021; Comerota et al., 2019).

This light-induced autophagy stimulation should sort the optimal effects right where the light quanta directly impact autophagy-sensitive substrates. In fact, the macular region is open to the impact of natural light without any neural or blood structure. At this level, the concentration of light produces its maximal effects on the stimulation of the autophagy machinery.

At this point, a natural question rises on whether light-induced autophagy and autophagy stimulation by phytochemicals may synergize upstream. In other words as reported in the following paragraph how inert phytochemicals are to the effects of light? Is it possible that light stimulation may enhance the autophagy induction induced by phytochemicals?

Light-nutrients interaction may enhance the autophagy stimulation

As macular region directly receives the light quanta and their concentration is the highest considering the whole retinal surface unit, one should consider that even the concentration of phytochemicals administered systemically may specifically focus in the macular region. In fact, this is the case of lutein, which is mostly stored in the macula lutea. In this scenario, it is tempting to analyze whether the chemical properties of lutein are modified by the impact of a physical source such as light to sort improved biochemical effects (Figure 2). In fact, specific wave-lengths determine a change in the structure and chemical properties of lutein (Aziz et al., 2020). In keeping with the three nutrients administered in the Lugano protocol also the structure of resveratrol is modified by light exposure (Lu et al., 2021). Even in the case of bilberry, light alters the composition of this mixture of phenolic compounds by generating alternative metabolites, still belonging to polyphenols (Karppinen et al., 2016).

This recent evidence suggests that adding phytochemicals and related natural compounds to the natural light is not simply synergizing the induction of autophagy machinery based on common downstream effects. Such an association owns an intrinsic value, which is based on the fact that, natural nutraceuticals, and natural light interacts to enhance their biochemical effects in the cells (Figure 2). These effects are key in preserving the functional and structural integrity of the retina and they are likely to provide a remedy to counteract retinal degeneration and in sustaining vision.

In fact, a number of nutraceuticals derives from light sensitive plants and the interaction with light is key to modulate these compounds to exert their trophic effects. In this way, the reason of combining light with nutraceuticals is rooted within the archaic nature of the interactions between light quanta and specific ancestral biochemical species. The combined treatment of light and food, which at first glance appear as a rough approach is indeed the recapitulation of the evolutionary role of these stimuli, which converge mutually, before converging on their common targets in the autophagy pathway. In fact, light is needed to promote survival of plants and to drive differentiation acting on chemical species, which are shaped as a result of light exposure. In this way, combining light and nutraceutical according to specific gold-standard protocols may be considered as a whole merged natural stimulation to provide retinal integrity, evolution and survival.

Exploring other autophagy-driven mechanisms which may provide a synergism of photobio-modulation in restoring retinal integrity

Although autophagy seems to cover most pathological and biochemical effects, which are key in sustaining retinal integrity and in counteracting retinal degeneration, it is unlikely that organelles and molecules removal alone may be entirely responsible for the beneficial effects provided by light and nutraceuticals as they were described so far. The synergism between electromagnetic field and

chemical moyeties in promoting the anatomy of the retina may extend to other target and mechanisms.

This field involves the recent evidence that specific wave-lengths tune the proliferation and the differentiation of specific stem cell niches in the retina. These groups of stem cells are highly conserved in the adult retina from rodents to humans. They are placed at various level in the adult retina. In keeping with this, alternative effects produced by autophagy activation include sub-serving specific architectural planning to achieve adaptive functions. This is in line with the occurrence of an autophagy damage as a trigger phenomenon in producing a number of chronic and acute retinal diseases (Pinelli et al 2020b, 2020a; Intartaglia et al., 2021). The classic role, which is attributed to the autophagy machinery consists in removing a variety of substrates through their entrapment within specific organelles named autophagosomes. In this way, the essential definition of autophagy is to provide the clearance of misfolded proteins, lipids, sugars and damaged organelles including mitochondria. These molecules and organelles in turn are aggregated within stagnant lysososmes within retinal cells, when a fast or slow retinal degenerative disorders takes place (Blasiak et al. 2014, Nita et al. 2020, Bilbao-Malavé et al. 2021, Yako et al. 2021).

Apart from counteracting retinal injury, the process of autophagy was recently hypothesized to be fundamental in fostering regenerative phenomena in the retinal layers. These include both maladaptive and beneficial maturation phenomena, which take place following either chronic or acute retinal injuries. The involvement of autophagy is recently postulated to cover the mechanisms of regeneration, which occurs as maturation phenomena following chronic degenerative conditions and sudden injuries. In fact, in the diseased retina just like a number of areas within the CNS disorders an altered autophagy is seminal to promote chronic degenerative conditions (Ravikumar and Rubinsztein, 2004; Rubinsztein et al., 2005; Fornai et al., 2008a, 2008b; Castino et al., 2008; Ferrucci et al., 2008; Isidoro et al., 2009; Madeo et al., 2009). Similarly, autophagy is altered during maturation phenomena which take place over time following acute neurodegeneration such as those happening following brain intoxication, prolonged epileptic seizures, hypoxia and ischemia (Giorgi et al., 2015; Lazzeri et al., 2021; Wang et al., 2022a, 2022b; Zhang et al., 2021; Biagioni et al., 2022; Xiao et al., 2021).

This happens also within brain areas, which are placed around a traumatic injury which may or may not recover partly depending on the amount of ongoing autophagy (Arruri and Vemuganti, 2022; Chen et al., 2022).

In fact, such a peri-traumatic region may be viewed as a sort of traumatic penumbra analogous to the ischemic and epileptic penumbra, where cells are on the edge of their viability depending on deleterious or beneficial influences. The latter seem to be fostered by an effective autophagy status, which removes cell substrates being altered by the traumatic injury. Recent studies suggest that in these degenerating regions autophagy may promote recovery either acting on dying cells or promoting the proliferation and differentiation of specific stem cell niches (Chang et al., 2020). In this scenario autophagy sustains cell proliferation and cell differentiation towards the required neuronal phenotypes. These effects of autophagy on stem cell niches at retinal level may be critical in combining the effects of light and nutraceuticals. The stem cell-rich site in the retina occurs at the retina-choroid junction in the retinal development. This corresponds to an area, which is mostly involved during the course of retinal degeneration. Thus, it is fascinating that stem cell niches in the retina mostly occurs in sites, which are key targets for retinal disorders just like the junction between choroid and retina. Some stem cell properties were described concerning the retinal pigment epithelium RPE (Salero et al., 2012, Bernstein et al. 2020). In fact, the plasticity of the RPE depends on the presence of melanin and the sympathetic innervation. In some animal species the plasticity of RPE may lead to the development of the retina as well as the lens. Such an evolutionary potential can be rescued during adult life concerning a specific sub-set of RPE cells, which maintain their proliferation upon specific stimuli. In these cases, RPE cells may regenerate both neural and mesenchymal cells distributing to both sides of the retinal border, which implies their invasion of the choroid or their course towards inner retinal layers. Such a subset of RPE may be involved in specific disorders, where a combined pathology of the retina and corpus vitreum takes place (Salero et al., 2012). In keeping with the cell types mentioned as key in

the process of retinal degeneration, Muller cells do possess stem cell properties (Bhatia et al., 2009, 2010; Das et al., 2006; Zhao, et al., 2014; Singhal et al., 2012). It is now evident that Muller cells represent a sort of dormant retinal progenitor stem cells, which are sensitive to the inhibitory effects of epinephrine (Zhu et al., 2021). Upon retinal degeneration these cells are able to differentiate and migrate towards various retinal layers. The geographical areas, where the occurrence of stem cells is mostly evident involves the segment between the ora serrata and ora terminalis (Nickerson et al., 2007, Bhatia et al., 2009; 2010, Aladdad et al., 2019; Coles et al., 2004; Das et al., 2006); the iris pigment epithelium (Seko et al., 2012,) and the region where the optic nerve emerges (Bernstein et al., 2020). Within these geographical areas the MC and the RPE remain the two main sources of stem cells. At this level, both specific wave-lengths and nutraceutical do exert stimulatory effects. The effects of light in stimulating stem cell is not unique for the retina since near infra-red light of 808 nm activates hippocampal stem cells when applied at 20 mW/cm2 (Guo et al. 2021). The preferential placement of the retina to the stimulatory effects of light are supposed to foster such an effect as shown by the stimulating effects of red light in inducing stemness from astrocytes (Yoon et al., 2021; Chang and Lee, 2021). In line with this, Shiraya et al. (2022) recently demonstrated a marked stimulation of specific genes after exposure of retinal stem cells from the RPE to specific wave-lengths produced by a laser source. Among these genes, autophagy-related chaperonins, like the heat shock protein gene family was induced. This is expected to have a profound impact on eye stem cells, where a concomitant activity of nutraceuticals was recently hypothesized.

Conclusions

Autophagy is effective in sustaining the viability of the whole retina although the outer retina and the choroid-retinal border critically relies on effective autophagy flux. Among these anatomical structures, the RPE is mostly influenced by the amount of ongoing autophagy, which is based on its physiological role in removing cell debris, sustaining the metabolism of photoreceptors, clearing the outer retina from damaged organelles and toxic chemical species. In addition, autophagy stimulation provides a powerful inducer for a sub-type of RPE cells which possess a stem cell activity. The present manuscript indicates how autophagy impairment may be the trigger to start retinal degeneration mostly concerning AMD, where further steps in disease progression including the shift from dry into wet AMD can be regulated by the autophagy machinery. The activation of autophagy by using natural stimuli such as pulsatile light exposure combined with specific nutraceuticals appear to be a powerful remedy to counteract or even reverse the pathology and visual loss occurring during AMD. The role of autophagy is discussed concerning several effects. Remarkably, the removal of the pathological hallmarks of AMD, so-called drusen, may be concomitant to a specific biochemical effect, which sustains the process of vision. This may explain why autophagy ameliorates both drusen formation and visual acuity as concomitant rather than causally associated events. The use of natural sources of activation of the autophagy machinery in the retina may represent the natural mimicking of a remedy, which does not bring the burden of severe side effects when reiterated for long time intervals.

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