ISSN 2079-0570, Advances in Gerontology, 2017, Vol. 7, No. 1, pp. 17–24. © Pleiades Publishing, Ltd., 2017.
Original Russian Text © D.V. Telegina, O.S. Kozhevnikova, N.G. Kolosova, 2016, published in Uspekhi Gerontologii, 2016, Vol. 29, No. 3, pp. 424–432.

Molecular Mechanisms of Cell Death in Retina during Development of Age-Related Macular Degeneration

D. V. Telegina, O. S. Kozhevnikova, and N. G. Kolosova

Federal Research Center Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk, 630090 Russia

e-mail: kolosova@bionet.nsc.ru

Abstract—Age-related macular degeneration (AMD) is a chronic progressive disease characterized by damage to the central retina zone. Changes in choriocapillaries, retinal pigment epithelium (RPE), and Bruch's membrane (typical for aging) underlie AMD pathogenesis; however, the mechanisms launching the transfer of typical age-related changes in the pathological process are unknown. The death of photoreceptors and irreversible loss of vision become the results of pathological changes in RPE and choroid. In spite of intensive studies of AMD pathogenesis, information on molecular genetic preconditions of the events leading to the death of photoreceptors (as well as about the pathways of their death) is extremely limited; this complicates the search for efficient methods of AMD treatment (first of all, the most widely spread atrophic ("dry") form of the disease). Recent studies demonstrated that not only apoptotic but also autophagic and necrotic signaling cascades are involved in the cellular death of retinal cells. The published data on three main forms of the programmed cell death (apoptosis, necrosis, and autophagy) and their role in AMD pathogenesis are summarized in the present review.

Keywords: age-related macular degeneration, retina, apoptosis, necroptosis, autophagy

DOI: 10.1134/S2079057017010155

INTRODUCTION

Age is a leading risk factor for the development of age-related macular degeneration (AMD), a complex disease, which is the main reason for irreversible loss of vision in individuals older than 60. AMD morbidity is increasing against a background of an increase in lifespan. According to predictions, the number of individuals suffering from AMD will increase by a third by 2020 [30] and in the United States alone will significantly exceed 3 million [1]. AMD is a complex multifactorial disease leading to irreversible death of photoreceptors, subject cells of retinal pigment epithelium (RPE), changes of Bruch's membrane and choriocapillaries in the area of macula, and loss of central vision [29, 30]. "Dry" and "wet" forms of the disease are categorized. Approximately 90% cases are of the "dry" atrophic form of AMD; today, there is no method of its treatment [56]. In the "dry" form of AMD, drusen are diagnosed in the macular area, pigment redistribution occurs, defects of pigment epithelium and the choriocapillary layer appear, and death of photoreceptors occurs against a background of RPE cell atrophy [29]. "Wet" (exudative) form develops in ~10% of AMD patients and is characterized by ingrowing of newly generated vessels through Bruch's membrane defects under the retinal pigment epithelium or under neuroepithelium. The increased permeability of newly generated vessels results in retina edema, exudation, and hemorrhage in the vitreous body and retina (which finally becomes the reason for vision loss) [1, 29].

Changes typical for aging underlie the AMD pathogenesis, but the mechanisms launching the transfer of usual age-related changes in the pathological process are unknown. Cell-death intensification in tissues (including of the eye) occurs during aging; this favors the development of structural and functional damages. The existence of a connection between AMD pathogenesis and inflammation and oxidative stress, mitochondrial dysfunction, and changes of vessels has been proven; however, the specific mechanisms of the death of photoreceptors that result in the loss of vision have not been finally established and are being actively discussed [49, 69]. A significant portion of cells die by apoptosis, which, on the one hand, is a physiological process and plays a significant role in the development of eye and retina homeostasis, but, on the other hand, is one of the main mechanisms of pathological changes in affected organs and tissues. Convincing evidence that apoptosis is not the main form of the cell death during AMD has appeared in recent years.

BRIEF DESCRIPTION OF CELL-DEATH TYPES

Apoptosis, necrosis, and autophagy are the three main cell death types, the classification of which was suggested by J.U. Schweichel and H.J. Merker in 1973 based on ultrastructural studies of physiological cell death in prenatal tissues. Morphological characteristics of each cell death type are different. The first type (apoptosis) is characterized by the cytoplasm condensation, chromatin aggregation, and DNA fragmentation, generation of apoptotic bodies, decrease in the cell volume, and the cell rounding. The cells affected by apoptosis are recognized by macrophages or other phagocytes and rapidly eliminated avoiding the development of inflammatory reaction. The second cell-death type (necrosis) is characterized by swell of the cytoplasm and organelles, increase in cell volume, membrane breakage, and change in the intercellular-substance composition. The development of large inclusions (autophagosomes and autolysosomes) in the cytoplasm and absence of condensation and fragmentation of the cells are typical for the third death type (autophagy). In spite of some differences in criteria and nomenclature of the cell-death mechanisms, this classification has been accepted as the main one and has been widely used in the literature since 1970 [49].

Today, *apoptosis* is the most characterized form of cell programmed death. The signaling pathways that regulate it and its key components (apoptotic proteins) have been determined; however, the mechanisms of disturbance of their functions during the development of pathological processes are far from understanding. The term "apoptosis" most frequently indicates the caspase–dependent mechanism, which is characterized by successive activation of a number of cysteine proteases (caspases). There are two main pathways leading to caspase activation: an external pathway induced by the cell-death receptors and an internal pathway, which is regulated by mitochondria. The external apoptosis pathway is activated by means of transmembrane cell-death receptors that have an *N*-terminal extracellular domain (responsible for the binding to ligand) and a *C*-terminal intracellular part, which is used for apoptosis-signal transmission. Six main types of receptors are known: *TNFR*1, *CD*95, *DR*3, *TRAILR*1, and *TRAILR*2 [37]. They are all characterized by the presence of the highly conservative intracellular cytoplasmic domain ("death domain") required for signal transduction consisting of approximately 60–80 amino acids. The generation of multiprotein death-inducing signaling complex (*DISC*) occurs during the interaction between the death receptor and extracellular ligand [60]. Two types of *DISC* complexes are best studied. The first type is typical for the *FAS*, *TRAILR*1, and *TRAILR*2 receptors [37]: oligomerization of the receptor, which *Fas*-adaptor protein (*FADD*) binds to through homotypic death domains, occurs during the interaction with ligand. The region called the "death-effector domain" (*DED*), which binds similar procaspase-8 (procaspase-10 in human) domain, is located at the *FADD* N-terminus (thus developing *DISC*) [70]. The second *DISC* complex is linked to *TNF*-mediated apoptosis and has a number of distinctive features in the molecular mechanism from the signaling pathway of *FAS* and *TRAILR*1/*R*2 receptors. The *TNFR*1 receptor stimulation results in generation of two signaling *DISC* complexes (complex I and complex II (*A*/*B*)). After binding to the ligand, the *TNFR*1 receptor binds to the protein associated with the death domain of *TNF* (*TRADD*) receptor and *RIP*1 bound to *TNFR* factors 1 and 2 (*TRAF*-1/2), cellular-apoptosis inhibitor (*cIAP*-1/2), and linear complex of ubiquitin ligase (*LUBAC*) (finally generating I *TNF-R*1 complex). Complex I can favor efficient activation of the *NF-JB* pathway [4]. The process of complex II development is not completely clear, but it is known that *RIPK*1 and *TRAF*1 dislocation from complex I occur, increasing the *TRADD* release in the cell cytoplasm and its association with *FADD* through the death domains. The *FADD* binds to procaspase-8 according to the mechanism described above, generating complex II (*TRADDosome*). Both *DISC* complexes activate caspase-8. Activated caspase-8 is able to cleave specific substrates in cytosol, particularly effector procaspases (caspase-3, -6, -7), which finally results in the launch of the apoptosis mechanism (discussed in more detail in reviews [60], [70]).

Cellular stress, DNA damage, activation of oncogenes, hypoxia, oxidative stress, and irradiation promote the activation of the internal apoptosis pathway. These stimuli lead to a change in mitochondrial-membrane permeability and release of apoptotic proteins from the intermembrane space of mitochondria in the cell cytoplasm [63]. The integrity of the mitochondrial membrane is regulated by a balance between proapoptotic and antiapoptotic proteins from the *Bcl*-2 family. When proapoptotic signal activation in response to internal or ecological stress exceeds a certain threshold value, *Bcl*-2-associated *X* protein (*Bax*) is inserted into the mitochondrial membrane and is oligomerized, generating channels on its outer membrane [8]. This results in an increase in the permeability of the mitochondrial outer membrane and release of cytochrome *c* and secondary mitochondrial activator of caspases (*Smac*) from intermembrane space in the cytosol. Cytochrome *c* binds to apoptosis protease-1 activating factor (*Apaf*-1), inducing its conformational changes and oligomerization. The *Apaf*-1–cytochrome *c* protein complex (called "apoptosome") is dimerized and mediates the conformational change and activation of initiator caspase-9. In turn, activated caspase-9 cleaves the effector procaspases-3 and -7 that subsequently cleave most of cellular proteins, including the cytoskeleton proteins [46]. Both external and internal apoptosis pathways lead to the change in

the outer mitochondrial membrane permeability. Mitochondria are rich with proapoptotic proteins, and the change in external mitochondrial membrane permeability is a irreversible step during the cell-death launch [8, 63].

It was considered that *necrosis* is a result of excessive cytotoxic or mechanical damage and has no specific molecular mechanisms. However, conclusive data have been accumulated in the last decade that cellular signaling pathways initiate necrosis not by chance, but in response to specific signals. The conception of programmed necrosis (or necroptosis) is discussed in the literature more than a year; the works that expanded our understanding of the mechanisms and regulation of this process conducted by means of serine/threonine kinases (*RIP*) and pseudokinase substrate (*MLKL*) appeared recently [31]. *RIP* kinases play a central role in necroptosis signal transduction: *RIPK*1 and *RIPK*3 are not cleaved and can interact through *Rhim* domains (functioning as kinases) during the inhibition of caspase-8 in the complex II *DISC* at *TNF*-mediated apoptosis. *RIP*1 and *RIP*3 together with *MLKL* are involved in the development of necrosome (a complex, which includes the *FADD*, *MLKL*, caspase-8, *RIP*1, and *RIP*3) [19]. In necroptosome, the *RIP*1 phosphorylates *RIP*3, which in turn phosphorylates *MLKL* inducing oligomerization of *MLKL* pseudokinase, which is then translocated to the plasma membrane. The further role of *MLKL* is not completely clear: on the one hand, it was demonstrated that *MLKL* favors the inflow of Ca^{2+} [6] and Na^{+} [7] ions in the cell, which increases the osmotic pressure and leads to the membrane rupture and subsequent death; on the other hand, data exist according to which oligomerized *MLKL* binds membrane lipids through positively charged amino acids at its *N*-terminus, resulting in the development of pores on the membrane and disturbance of its integrity [66].

Autophagy is an intracellular process of degradation of cytoplasmic components in lysosomes that maintains cellular homeostasis and energy production. It is used not only for degradation of damaged organelles and long-living proteins, but also as a recirculating dynamic system: autophagy is required for the maintenance of homeostasis and metabolic adaptation. Three main types of autophagy are distinguished: microautophagy, macroautophagy, and chaperone-mediated autophagy (*CMA*) [48]. *CMA* was only described in mammals and is involved in degradation of certain soluble proteins, while micro- and macroautophagy are present in most eukaryotes (including mammals, plants, and fungi) and provide degradation of parts of the cytoplasm with cellular organelles [48].

Microautophagy is a nonselective process of degradation; its precise physiological functions in mammals are not completely clear. The cytosol material at microautophagy is directly captured by invaginations of the lysosome membrane. The membrane dynamics is very similar to *ESCRT* (sorting complex of endosomes required for the transport) [58]. It is believed that the maintenance of the organelle size, membrane homeostasis, and providing cell survival while limiting the amount of nitrogen is the main function of microautophagy [40].

Chaperone-mediated autophagy is characterized by the absence of membrane reorganization, and directed transport of partially denatured proteins from cytoplasm through the lysosome membrane in its cavity occurs. The *hsc70* heat-shock protein with the involvement of cochaperones specifically recognizes cytosol proteins containing the *KFERQ* pentapeptide or its analogs [9]. It is believed that the acquisition of *KFERQ* pentapeptide by the target protein is one of the stages of posttranslational protein modification. The complex of *hsc70* with the target protein is delivered to the lysosome membrane, where it binds to the transmembrane *LAMP2A* protein acting as a receptor on the lysosome. The second chaperone (lysosomal *hsc70*, *lys-hsc70*) contributes to the displacement of the target protein in the lysosome clearance, where it is completely destroyed by proteases [39]. This type of autophagy is distinguished by selectivity of degraded proteins and direct translocation of the target proteins through lysosomal membranes without the development of additional vesicles.

Macroautophagy is the most studied autophagy pathway (subsequently, autophagy). In spite of the fact that autosomal structures were already found by means of electron microscopy in the 1950s, ideas on the molecular mechanism of autophagy began to form only in the 1990s after the discovery of autophagy-dependent genes (*Atg*) in yeasts [51, 59]. Of the 40 genes encoding the *Atg* proteins, 15 were also found in higher mammals (*Atg*1–*Atg*10, *Atg*12–*Atg*14, *Atg*16, and *Atg*18) [48]. The autophagy process is usually divided into five stages: 1, initiation; 2, generation of double membrane and development of autophagosome; 3, elongation; 4, fusion with lysosome; and 5, degradation of absorbed components [35]. It is considered that initiation of autophagy occurs during the generation of isolating membrane (called phagophore) in close proximity to endoplasmic reticulum. The studies demonstrate that different organelles, including endoplasmic reticulum, canalicular apparatus (Golgi apparatus), mitochondria, plasmatic membrane, and endosomes, are a substrate for the development of phagophores [35, 48].

Two protein kinase complexes are required for initiation of the autophagy process. The first *ULK*1 complex (consisting of the *ULK*1, *Atg*13, and *FIP*200 proteins) suppresses the *mTOR* complex 1 (*mTORC*1), which is the main negative regulator of autophagy. The *mTORC*1 inhibition results in the activation of the *ULK*1 complex, which promotes the development of the second protein kinase complex (class III *PI* complex, consisting of the *Beclin* 1, *Atg*6, *Atg*14, *Vps*15,

*Vps*34, and *Ambra*1 proteins. This kinase complex promotes the generation of *PI3P* lipids (phosphatidylinositol 3-phosphate) required for the development of vesicles [59]. Two ubiquitin-like reactions occur during the elongation stage. Several *Atg* proteins (*Atg*7, *Atg*10, *Atg*5, and *Atg*12) are involved in the first reaction. *Atg*7 and *Atg*10 have an enzymatic activity (*E1*- and *E2*-enzyme, respectively) and act as a "label" for the material to be deleted [51]. The attachment of the *Atg*12 protein to its substrate (*Atg*5 protein) occurs after the material recognition, and then *Atg*16*L1* multimerization occurs on the *Atg*5–*Atg*12 complex. The developed protein complex is associated with the phagophore generating autophagosome. Subsequently, a second ubiquitin-like reaction occurs: the *Atg*7 (*E1*-enzyme) and *Atg*3 (*E2*-enzyme) enzymes promote the activation and attachment of ubiquitin-like molecules of the *Atg*8 family (the *LC*3 is the best known in mammals) to the autophagosome membrane due to the membrane modification and generation of lipid "anchor" [51]. The *Atg*8 protein is associated with the cytoskeleton microtubules, due to which the autophagosome movement and expansion occurs for subsequent fusion with lysosome or endosome. The separation of the *Atg*5–*Atg*12–*Atg*16*L1* complex occurs during the closing of autophagosome; however, the *LC*3-II protein (lipid *LC*3 form) remains bound to autophagosome before its fusion with lysosome [51, 59]. Thus, the *LC*3 can be used as a protein marker of autophagosomes.

Autophagy can be regulated by many diverse factors and can be induced by different stimuli—limitation of nutrients, hypoxia, and active oxygen forms—as well as the presence of protein aggregates and damaged organelles.

THE ROLE OF APOPTOSIS, NECROPTOSIS, AND AUTOPHAGY IN PATHOGENESIS OF AGE-RELATED MACULAR DEGENERATION

The idea that which apoptosis is the main mechanism of the cell death during induced or hereditary retina degeneration was formulated in the first decade of the 2000s [22, 25, 53–55]. The loss of visual cells by apoptosis as a key characteristic of AMD was described by C.E. Remé et al. [53–55], while C.A. Curcio et al. demonstrated that the death of mainly cones (but not rods) occurs at AMD [10, 11]. These authors suggested that degeneration and loss of photoreceptors outruns in time dysfunctions in the complex of RPE and Bruch's membrane [11]. The apoptosis of photoreceptors, RPE, and internal nuclear-layer cells in postmortem retina samples in patients with AMD was later described, and it was suggested that the *FasL*/*Fas* system is involved in apoptosis [17]. The presence of *TUNEL*-positive cells in RPE and photoreceptor layer at the edges of atrophic regions (with the appearance of which the loss of vision in patients with geographical atrophy is associated) was detected [17]. H. Maeda et al. demonstrated that apoptotic loss of photoreceptors in ornithine-induced degeneration is directly associated with RPE cell death. These results indicate the important role of RPE cells in the maintenance of viability of photoreceptors [44].

It was demonstrated that the death of rods in the retina is characterized by chromatin fragmentation; however, the involvement of caspases depends on the age and type of disease. During the development of retina, the activation of caspases passes through the mitochondrial pathway as indicated by a high expression of proapoptotic genes from the *Bcl*-2 family (such as *Bax*, *Bak*, *Bim*, and *Puma*), as well as the *Apaf*-1 and caspase-3 in the retina at early stages of development [22]. With age, photoreceptors become more resistant to proapoptotic stimuli and most likely die on the caspase-independent pathway [16]. According to a number of authors, classical caspase-dependent apoptotic mechanism is suppressed after the completion of the development of retina. Photoreceptor cells are postmitotic. Several control of apoptosis is required for their survival, since rapid activation of caspases and cellular death will have serious consequences for mature retina. It is possible that activation of the caspase-independent apoptosis pathway through alternative proteases (cathepsins, granzymes, and calpains) occurs during the retina degeneration in response to stress [16]. The question of the role of apoptosis, as well as its mechanism (caspase-dependent or caspase-independent pathway) during AMD, remains controversial and is being actively studied [69].

Studies of animals with photoinduced retinal damage have contributed significantly to the understanding of the mechanisms of apoptosis regulation under AMD [68]. The excessive light effect is able to accelerate the development and increase the severity of AMD [52], as well as some forms of pigment retinitis in humans [45]. The existence of increased susceptibility to light retina damage in animals (models of hereditary retina degeneration) has been proven [36]. The excessive effect of white light induces apoptosis of photoreceptors, thus providing an available model for the analysis of this process mechanisms [25]. It is logical that the process of apoptosis of photoreceptor cells depends on the light intensity and, probably, on specific biological models [68]. Thus, an increase in the mRNA level of several caspases is observed at light intensity of the effect [52]. The caspase-3 mRNA level was increased in the retina in rats after 6–12 h of light effect, but the caspase-3 enzymatic activity did not change [62]. The caspase-3 activity was not increased under light-induced retina degeneration [38], while the activity of calcium-dependent calpains increased, promoting the blocking of calcium channels and suppression of photoreceptor apoptosis [16]. An increase in the activity of caspase-3, as well as cathepsin and lysozyme (involved in autophagy), has been found after moderate light damage [43].

The attractive hypothesis was gradually developed that apoptosis is the main cellular-death pathway during the development of AMD, while apoptosis inhibition can become a promising therapeutic approach to the disease treatment. However, attempts at pharmacological inhibition of caspase pathway did not prevent the loss of cells during retina detachment [49]. Moreover, it was established in a extensive study of retina degeneration on ten biological models that correspond to all main groups of human hereditary blindness (*rd*1, *rd*2, *rd*10, *Cngb*1 *KO*, *Rho KO*, *S334ter*, *P23*H, *Cnga*3 *KO*, *cpfl*1, *Rpe*65 *KO*) that apoptosis plays an insignificant role in cell death during these diseases. At the same time, the authors demonstrated that alternative cellular death mechanisms play a significant role in hereditary retina degeneration [3].

Lately, data on the role of necrosis in the retina pathology have been accumulated [26, 61, 66]. It was demonstrated that the conditions are not optimal for the launch of apoptosis during ischemic retina damage and are more suitable for the induction of the programmed necrosis (necroptosis) [12]. Some researchers assume that necroptosis can be the main cellular-death pathway in the retina after different types of damage [18, 21, 28]. Thus, necroptosis becomes a cause of cell death and damage of neurons during experimental retina detachment [13, 15]. This hypothesis is supported by the fact that necrostatin-1 (a specific necroptosis inhibitor) decreases neuronal cell death after the retina detachment [14]. *RIP* kinase also promotes necrotic cell death during the inhibition of the caspase apoptosis pathway (suppressing apoptosis) [64]. The *RIP*3 (one of the main necroptosis regulators) is involved in an ischemic reaction in response to stress in the retina [42]. It has been demonstrated on blind *pde*6c fishes (*zebrafish*) that necroptosis is the main mechanism of cone death, while rods die by means of caspase-dependent apoptosis [65].

At present, RPE is in the spotlight of researchers of the cell-death mechanisms in the retina, since a hypothesis exists that its cells promote the development of chronic inflammatory processes [2] (that play an important role in AMD pathogenesis) [27]. RPE cells are postmitotic and, therefore, have a limited apoptotic potential, while necrosis (as opposed to apoptosis) can induce inflammatory and immune responses. Recently, it was demonstrated on the mouse model of *dsRNA*-induced retina degeneration that *RIPK3*-mediated secretion of *HMGB*1 in the vitreous body correlated with RPE cell necrosis and production of *TNF*-α and *IL*-6 [50]. These results demonstrate that RPE cell necrosis in response to oxidative stress has a negative effect during tinflammation.

It has been established that necroptosis is the main pathway of RPE cell death (*ARPE*-19) in the culture in response to oxidative stress [26]. Under conditions of acute oxidative stress caused by the introduction of sodium iodate ($NaIO_3$) with selective toxicity for RPE cells, the latter were also exposed to necrosis, which was followed by apoptosis of photoreceptors and retina thinning [67]. Using another model of oxidative retina damage (mouse lines deficient for the ceruloplasmin ferroxidase (*Cp*) and hephaestin (*Heph*) genes), it was demonstrated that structural–functional damage and RPE cell necrosis occur in this animals at the age of 6–9 months and preceded subretinal infiltration of macrophages and death of photoreceptors that occur at the age of 12 months [23, 24]. The swelling and vacuolization of RPE cells (typical for necrosis and necroptosis) are observed in several mouse AMD models, including in the *Ccl2* – / – /*CX*3*cr*1 – / – mice (Chu et al., 2013; Mattapallil et al., 2012) and in knockout mice for the *apoE*2 and *apoE*4 on a diet with high content of fats [27]. These data indicate in favor of the fact that necrosis can play a significant role in RPE cell death during the development of neurodegenerative processes in the retina.

There is still no common opinion about the mechanism of RPE cell death during the development of AMD in individuals; the discussion between supporters of the dominant role of apoptosis and necrosis continues [27]. This is probably caused by the fact that a significant part of studies was carried out earlier than necroptosis was characterized as a regulated cell-death pathway. In addition, due to slow progression of AMD (5–15 years) it is difficult to track the events occurring in RPE cells during the development of the disease. Furthermore, we usually have to work with postmortem samples (that necessitates interpreting the results of the study of retina in patients with AMD with great caution). Nevertheless, based on data on the character of RPE cell death in vitro, results of studies on mouse AMD models, and histological retina preparations in patients, J. Hanus et al. suggested a hypothesis according to which necrosis is the main mechanism of RPE cell death during the development of AMD. These authors suggest that oxidative stress and/or other stressors induce the necrosis and RPE cell dysfunction, that in turn results in the development of drusen, activation of components of complement and immune response and, as a consequence, to the death of photoreceptors (more details in review [27]).

The dysfunction of autophagy with age in postmitotic cells (including RPE cells) can lead to the accumulation of damaged proteins in them and, as a consequence, to the degradation and cell death [33]. It is logical that accumulation of protein aggregates as a result of disturbances in the autophagy processes is a common feature of pathogenesis of AMD and other neurodegenerative diseases associated with aging [34]. In addition, hypoxia, oxidative stress, and inflammation act as the autophagy inductors [5]. The proteolytic activity compensatorily increases in cells with age due to an increase in the number of damaged proteins and organelles that should be removed. Recent studies by N. Rodriguez-Muela et al. demonstrated changes of proteolytic potential in the retina in *C*57*BL*6 mice

with age. According to their data, the activity of macroautophagy significantly decreases with age, which is accompanied by the appropriate increase in the activity of chaperon-mediated autophagy, particularly an increase in the level of *Lamp2A* and *Hsc*70 proteins [57]. In addition, a disturbance in the mechanism of macroautophagy occurs at the stage of the development of autophagosomes, but not at the stage of component degradation.

It is logical that the studies on the role of autophagy in the pathogenesis of AMD are mainly focused on the study of this process in RPE cells that perform a number of functions important for the retina, including phagocytosis of external segments of photoreceptors. Throughout life, RPE is exposed to a high oxygen tension and bright lighting (maintaining the capability for phagocytosis and removal of degradation products) [41]. The processes of autophagy are intensified in RPE cells under normal aging or at the beginning of the development of AMD (compensating for an intensification of oxidative stress and increase in the number of damaged proteins and organelles) [47]. An increase in the autophagy markers (*LC*3, *Atg*7, and *Atg*9) in RPE cells and retina neuronal layers was detected in two mouse AMD models and in retina samples from patients with AMD. An increase in the level of *Atg*12–*Atg*5 and *LC*3 proteins in the Bruch's membrane and RPE was registered in old mice [33]. At present, there is convincing evidence that disturbance in autophagy leads to accumulation of lipofuscin and RPE cell damage (typical for AMD) [33, 47]. It is supposed that the character of changes in the process of autophagy depends on the stage of disease. RPE cells become "overloaded" by proteolytic activity during the disease progression; this leads to a decrease in the level of autophagy marker proteins (*LC*3, *Atg*7, and *Atg*9) [47]. It is also possible that the activity of lysosomal enzymes is suppressed due to the excessive accumulation of lipofuscin and RPE with age [20, 47]. Accumulation of *SQSTM*1/*p62* was found in RPE in patients with AMD in the yellow spot; its degradation occurs by autophagy, from which it follows that accumulation of *SQSTM*1/*p62* can indicate the suppression of this process [32]. It is obvious that both accumulation of lipofuscin and increase in the sensitivity to oxidative stress, disturbance of mitochondrial and lysosomal regulation (typical for AMD) can be associated with disturbances of the autophagy process. Nevertheless, it remains unclear whether the autophagy imbalance is a reason or consequence of the disease or these changes reflect the imbalance in the development and elimination of autophagosomes [20].

CONCLUSIONS

The evidence for a programmed character of biochemical changes leading to necrosis and the results of studies of molecular autophagy cascade resulted in a reconsideration of ideas about the mechanisms of the retina cell death during age-related macular degeneration. According to modern ideas, it is realized both by apoptosis and necrosis and autophagy. It is suggested that these processes take place simultaneously, disregulation in one pathway induces disturbances in others. All three forms of\cell death have a number of common mechanisms of regulation, the knowledge about the interaction of which during the retina aging and the development of age-related macular degeneration is extremely limited. In spite of intense studies on the pathogenesis of age-related macular degeneration, the information of molecular genetic preconditions of the events leading to the death of photoreceptors, as well as on the pathways of their death, is extremely limited; this complicates the search for efficient methods of treatment of age-related macular degeneration (first of all, the most widespread atrophic ("dry") form of the disease).

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project no. 15-04-02195) and by Russian Academy of Sciences (project no. 0324-2015-0023).

REFERENCES

1. Akpek, E.K. and Smith, R.A., Overview of age-related ocular conditions, *Am. J. Managed Care*, 2013, vol. 19, no. 5, pp. s67–s75.
2. Ardeljan, D. and Chan, C.C., Aging is not a disease: distinguishing age-related macular degeneration from aging, *Progr. Retinal Eye Res.*, 2013, vol. 37, pp. 68–89.
3. Arango-Gonzalez, B., Trifunović, D., Sahaboglu, A., et al., Identification of a common non-apoptotic cell death mechanism in hereditary retinal degeneration, *PloS One*, 2014, vol. 9, no. 11, p. e112142.
4. Ashkenazi, A. and Salvesen, G., Regulated cell death: signaling and mechanisms, *Ann. Rev. Cell Dev. Biol.*, 2014, vol. 30, pp. 337–356.
5. Blasiak, J., Petrovski, G., Veréb, Z., et al., Oxidative stress, hypoxia, and autophagy in the neovascular processes of age-related macular degeneration, *BioMed Res. Int.*, 2014, art. ID 768026.
6. Cai, Z., Jitkaew, S., Zhao, J., et al., Plasma membrane translocation of trimerised MLKL protein is required for TNF-induced necroptosis, *Nat. Cell Biol.*, 2014, vol. 16, no. 1, pp. 55–65.
7. Chen, X., Li, W., Ren, J., et al., Translocation of mixed lineage kinase domain-like protein to plasma membrane leads to necrotic cell death, *Cell Res.*, 2014, vol. 24, no. 1, pp. 105–121.
8. Choudhury, J.D., Kumar, S., Mayank, V., et al., A review on apoptosis and its different pathway, *Int. J. Biol. Pharmacol. Res.*, 2012, vol. 3, pp. 848–861.
9. Cuervo, A.M. and Wong, E., Chaperone-mediated autophagy: roles in disease and aging, *Cell Res.*, 2014, vol. 24, no. 1, pp. 92–104.

10. Curcio, C.A., Medeiros, N.E., and Millican, C.L., Photoreceptor loss in age-related macular degeneration, *Invest. Ophthalmol. Visual. Sci.*, 1996, vol. 37, no. 7, pp. 1236–1249.
11. Curcio, C.A., Photoreceptor topography in ageing and age-related maculopathy, *Eye*, 2001, vol. 15, no. 3, pp. 376–383.
12. Degterev, A., Huang, Z., Boyce, M., et al., Addendum: chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury, *Nat. Chem. Biol.*, 2013, vol. 9, no. 3, pp. 192–192.
13. Dong, K. and Sun, X., Targeting death receptor induced apoptosis and necroptosis: a novel therapeutic strategy to prevent neuronal damage in retinal detachment, *Med. Hypotheses*, 2011, vol. 77, no. 1, pp. 144–146.
14. Don, K., Zhu, H., Song, Z., et al., Necrostatin-1 protects photoreceptors from cell death and improves functional outcome after experimental retinal detachment, *Am. J. Pathol.*, 2012, vol. 181, pp. 1634–1641.
15. Dong, K., Zhu, Z.C., Wang, F.H., et al., Activation of autophagy in photoreceptor necroptosis after experimental retinal detachment, *Int. J. Ophthalmol.*, 2014, vol. 7, pp. 745.
16. Doonan, F., Groeger, G., and Cotter, T.G., Preventing retinal apoptosis—is there a common therapeutic theme? *Exp. Cell Res.*, 2012, vol. 318, pp. 1278–1284.
17. Dunaief, J.L., Dentchev, T., Ying, G.S., and Milam, A.H., The role of apoptosis in age-related macular degeneration, *Arch. Ophthalmol.*, 2002, vol. 120, pp. 1435–1442.
18. Dvoriantchikova, G., Degterev, A., and Ivanov, D., Retinal ganglion cell (RGC) programmed necrosis contributes to ischemia–reperfusion-induced retinal damage, *Exp. Eye Res.*, 2014, vol. 123, pp. 1–7.
19. Feoktistova, M. and Leverkus, M., Programmed necrosis and necroptosis signaling, *FEBS J.*, 2015, vol. 282, pp. 19–31.
20. Ferrington, D.A., Sinha, D., and Kaarniranta, K., Defects in retinal pigment epithelial cell proteolysis and the pathology associated with age-related macular degeneration, *Progr. Retinal Eye Res.*, 2015, vol. 51, pp. 69–89.
21. Gao, S., Andreeva, K., and Cooper, N.G., Ischemia-reperfusion injury of the retina is linked to necroptosis via the ERK1/2-RIP3 pathway, *Mol. Vision*, 2014, vol. 20, p. 1374.
22. German, O.L., Agnolazza, D.L., Politi, L.E., and Rotstein, N.P., Light, lipids and photoreceptor survival: live or let die? *Photochem. Photobiol. Sci.*, 2015, vol. 14, pp. 1737–1753.
23. Hadziahmetovic, M., Dentchev, T., Song, Y., et al., Ceruloplasmin/hephaestin knockout mice model morphologic and molecular features of AMD, *Invest. Ophthalmol. Visual Sci.*, 2008, vol. 49, p. 2728.
24. Hahn, P., Qian, Y., Dentchev, T., et al., Disruption of ceruloplasmin and hephaestin in mice causes retinal iron overload and retinal degeneration with features of age-related macular degeneration, *Proc. Natl. Acad. Sci. U.S.A.*, 2004, vol. 101, pp. 13850–13855.
25. Halaby, R., Does apoptosis regulate the function of retinal photoreceptors? *Med. Hypothesis, Discovery Innovation Ophthalmol. J.*, 2012, vol. 1, no. 2, pp. 21.
26. Hanus, J., Zhang, H., Wang, Z., et al., Induction of necrotic cell death by oxidative stress in retinal pigment epithelial cells, *Cell Death Dis.*, 2013, vol. 4, p. e965.
27. Hanus, J., Anderson, C., and Wang, S., RPE necroptosis in response to oxidative stress and in AMD, *Ageing Res. Rev.*, 2015, vol. 24, pp. 286–298.
28. Hisatomi, T., Sakamoto, T., Murata, T., et al., Relocalization of apoptosis-inducing factor in photoreceptor apoptosis induced by retinal detachment in vivo, *Am. J. Pathol.*, 2001, vol. 158, no. 4, pp. 1271–1278.
29. Horie-Inoue, K. and Inoue, S., Genomic aspects of age-related macular degeneration, *Biochem. Biophys. Res. Commun.*, 2014, vol. 452, no. 2, pp. 263–275.
30. Khan, M., Agarwal, K., Loutfi, M., and Kamal, A., Present and possible therapies for age-related macular degeneration, *ISRN Ophthalmol.*, 2014, art. ID 608390.
31. Khan, N., Lawlor, K.E., Murphy, J.M., and Vince, J.E., More to life than death: molecular determinants of necroptotic and nonnecroptotic RIP3 kinase signaling, *Curr. Opin. Immunol.*, 2014, vol. 26, pp. 76–89.
32. Kivinen, N., Hyttinen, J.M., Viiri, J., et al., Hsp70 binds reversibly to proteasome inhibitor-induced protein aggregates and evades autophagic clearance in ARPE-19 cells, *J. Biochem. Pharmacol. Res.*, 2014, vol. 2, pp. 1–7.
33. Klettner, A., Kauppinen, A., Blasiak, J., et al., Cellular and molecular mechanisms of age-related macular degeneration: from impaired autophagy to neovascularization, *Int. J. Biochem. Cell Biol.*, 2013, vol. 45, no. 7, pp. 1457–1467.
34. Kumar, S. and Fu, Y., Age related macular degeneration: a complex pathology, *Austin J. Genet. Genomic Res.*, 2014, vol. 1, pp. 5.
35. Lapierre, L.R., Kumsta, C., Sandri, M., et al., Transcriptional and epigenetic regulation of autophagy in aging, *Autophagy*, 2015, vol. 11, no. 6, pp. 867–880.
36. LaVail, M.M., Gorrin, G.M., Yasumura, D., and Matthes, M.T., Increased susceptibility to constant light in nr and pcd mice with inherited retinal degenerations, *Invest. Ophthalmol. Visual Sci.*, 1999, vol. 40, pp. 1020–1024.
37. Lavrik, I.N., Systems biology of death receptor networks: live and let die, *Cell Death Dis.*, 2014, vol. 5, no. 5, p. e1259.
38. Li, F., Cao, W., and Anderson, R.E., Alleviation of constant light-induced photoreceptor degeneration by adaptation of adult albino rat to bright cyclic light, *Invest. Ophthalmol. Visual Sci.*, 2003, vol. 44, pp. 4968–4975.
39. Li, W., Yang, Q., and Mao, Z., Chaperone-mediated autophagy: machinery, regulation and biological consequences, *Cell. Mol. Life Sci.*, 2011, vol. 68, pp. 749–763.
40. Li, W.W., Li, J., and Bao, J.K., Microautophagy: lesser-known self-eating, *Cell. Mol. Life Sci.*, 2012, vol. 69, pp. 1125–1136.
41. Li, W., Phagocyte dysfunction, tissue aging and degeneration, *Ageing Res. Rev.*, 2013, vol. 12, no. 4, pp. 1005–1012.
42. Liang X., Chen, Y., Zhang, L., et al., Necroptosis, a novel form of caspase-independent cell death, contributes to renal epithelial cell damage in an ATP-depleted

renal ischemia model, *Mol. Med. Rep.*, 2014, vol. 10, pp. 719–724.

43. Lohr, H.R., Kuntchithapautham, K., Sharma, A.K., and Rohrer, B., Multiple, parallel cellular suicide mechanisms participate in photoreceptor cell death, *Exp. Eye Res.*, 2006, vol. 83, pp. 380–389.
44. Maeda, H., Ogata, N., Yi, X., et al., Apoptosis of photoreceptor cells in ornithine-induced retinopathy, *Graefe's Arch. Clin. Exp. Ophthalmol.*, 1998, vol. 236, pp. 207–212.
45. Marigo, V., Programmed cell death in retinal degeneration: targeting apoptosis in photoreceptors as potential therapy for retinal degeneration, *Cell Cycle*, 2007, vol. 6, pp. 652–655.
46. Martinou, J.C. and Youle, R.J., Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics, *Dev. Cell*, 2011, vol. 21, no. 1, pp. 92–101.
47. Mitter, S.K., Song, C., Qi, X., et al., Dysregulated autophagy in the RPE is associated with increased susceptibility to oxidative stress and AMD, *Autophagy*, 2014, vol. 10, pp. 1989–2005.
48. Mizushima, N. and Komatsu, M., Autophagy: renovation of cells and tissues, *Cell*, 2011, vol. 147, pp. 728–741.
49. Murakami, Y., Notomi, S., Hisatomi, T., et al., Photoreceptor cell death and rescue in retinal detachment and degenerations, *Progr. Retinal Eye Res.*, 2013, vol. 37, pp. 114–140.
50. Murakami, Y., Matsumoto, H., Roh, M., et al., Programmed necrosis, not apoptosis, is a key mediator of cell loss and DAMP-mediated inflammation in dsRNA-induced retinal degeneration, *Cell Death Differ.*, 2014, vol. 21, pp. 270–277.
51. Noda, N.N. and Inagaki, F., Mechanisms of autophagy, *Ann. Rev. Biophys.*, 2015, vol. 44, pp. 101–122.
52. Organisciak, D.T. and Vaughan, D.K., Retinal light damage: mechanisms and protection, *Progr. Retinal Eye Res.*, 2010, vol. 29, pp. 113–134.
53. Remé, C.E., Grimm, C., Hafezi, F., et al., Apoptotic cell death in retinal degenerations, *Progr. Retinal Eye Res.*, 1998, vol. 17, pp. 443–464.
54. Remé, C.E., Grimm, C., Hafezi, F., et al., Apoptosis in the retina: the silent death of vision, *Physiology*, 2000, vol. 15, pp. 120–124.
55. Remé, C.E., Grimm, C., Hafezi, F., et al., Why study rod cell death in retinal degenerations and how? *Doc. Ophthalmol.*, 2003, vol. 106, pp. 25–29.
56. Rickman, C.B., Farsiu, S., Toth, C.A., and Klingeborn, M., Dry age-related macular degeneration: mechanisms, therapeutic targets, and imaging, *Invest. Ophthalmol. Visual Sci.*, 2013, vol. 54, no. 14, pp. ORSF68.
57. Rodríguez-Muela, N., Koga, H., García-Ledo, L., et al., Balance between autophagic pathways preserves retinal homeostasis, *Aging Cell*, 2013, vol. 12, pp. 478–488.
58. Sahu, R., Kaushik, S., Clement, C.C., et al., Microautophagy of cytosolic proteins by late endosomes, *Dev. Cell*, 2011, vol. 20, pp. 131–139.
59. Shimizu, S., Yoshida, T., Tsujioka, M., and Arakawa, S., Autophagic cell death and cancer, *Int. J. Mol. Sci.*, 2014, vol. 15, pp. 3145–3153.
60. Singh, N. and Bose, K., Apoptosis: pathways, molecules, and beyond, in *Proteases in Apoptosis: Pathways, Protocols, and Translational Advances*, New York: Springer-Verlag, 2015, pp. 1–30.
61. Szaboa, D.J., Toth, M., Doro, Z., et al., Cell death, clearance and inflammation: molecular crossroads and gene polymorphisms in the pathogenesis of age-related macular degeneration, *J. Biochem. Pharmacol. Res.*, 2014, vol. 2, pp. 132–143.
62. Tomita, H., Kotake, Y., and Anderson, R.E., Mechanism of protection from light-induced retinal degeneration by the synthetic antioxidant phenyl-N-tert-butylnitrone, *Invest. Ophthalmol. Visual Sci.*, 2005, vol. 46, pp. 427–434.
63. Tower, J., Programmed cell death in aging, *Ageing Res. Rev.*, 2015, vol. 23, pp. 90–100.
64. Trichonas, G., Murakami, Y., Thanos, A., et al., Receptor interacting protein kinases mediate retinal detachment-induced photoreceptor necrosis and compensate for inhibition of apoptosis, *Proc. Natl. Acad. Sci. U.S.A.*, 2010, vol. 107, pp. 21695–21700.
65. Viringipurampeer, I.A., Shan, X., Gregory-Evans, K., et al., Rip3 knockdown rescues photoreceptor cell death in blind pde6c zebrafish, *Cell Death Differ.*, 2014, vol. 21, pp. 665–675.
66. Wang, H., Sun, L., Su, L., et al., Mixed lineage kinase domain-like protein MLKL causes necrotic membrane disruption upon phosphorylation by RIP3, *Mol. Cell,* 2014, vol. 54, pp. 133–146.
67. Wang, J., Iacovelli, J., Spencer, C., and Saint-Geniez, M., Direct effect of sodium iodate on neurosensory retina, *Invest. Ophthalmol. Visual Sci.*, 2014, vol. 55, p. 1941.
68. Wenzel, A., Grimm, C., Samardzija, M., and Remé, C.E., Molecular mechanisms of light-induced photoreceptor apoptosis and neuroprotection for retinal degeneration, *Progr. Retinal Eye Res.*, 2005, vol. 24, pp. 275–306.
69. Wright, A.F., Chakarova, C.F., El-Aziz, M.M.A., and Bhattacharya, S.S., Photoreceptor degeneration: genetic and mechanistic dissection of a complex trait, *Nat. Rev. Genet.*, 2010, vol. 11, pp. 273–284.
70. Zamaraev, A.V., Kopeina, G.S., Zhivotovsky, B., and Lavrik, I.N., Cell death controlling complexes and their potential therapeutic role, *Cell. Mol. Life Sci.*, 2015, vol. 72, no. 3, pp. 505–517.

Translated by A. Barkhash