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Modes of Retinal Cell Death in Diabetic Retinopathy

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Abstract

Cell death seems to be a prominent feature in the progression of diabetic retinopathy. Several retinal cell types have been identified to undergo cell death in a diabetic environment. Most emphasis has been directed towards identifying apoptosis in the diabetic retina. However, new research has established that there are multiple forms of cell death. This review discusses the different modes of cell death and attempts to classify cell death of retinal cells known to die in diabetic retinopathy. Special emphasis is given to apoptosis, necrosis, autophagic cell death, and pyroptosis. It seems that different retinal cell types are dying by diverse types of cell death. Whereas endothelial cells predominantly undergo apoptosis, pericytes might die by apoptosis as well as necrosis. On the other hand, Müller cells are suggested to die by a pyroptotic mechanism. Diabetes leads to significant Müller cell loss at 7 months duration of diabetes in retinas of diabetic mice compared to non-diabetic, which is prevented by the inhibition of the caspase-1/IL-1β (interleukin-1beta) pathway using the IL-1 receptor knockout mouse. Since pyroptosis is characterized by the activation of the caspase-1/IL-1β pathway subsequently leading to cell death, Müller cells seem to be a prime candidate for this form of inflammation-driven cell death. Considering that diabetic retinopathy is now discussed to potentially be a chronic inflammatory disease, pyroptotic cell death might play an important role in disease progression. Understanding mechanisms of cell death will lead to a more targeted approach in the development of new therapies to treat diabetic retinopathy.

Keywords: Diabetic retinopathy; Cell death; Müller cells; Pyroptosis; Apoptosis; Necrosis; Inflammation

Introduction

The most common features of diabetic retinopathy are alterations to the retinal microvasculature leading to microaneurysms, macular edema, leakage of blood into the retinal tissue and vitreous, and eventual blindness [1,2]. Endothelial cells, which line the microvasculature and provide the blood-retinal barrier, have long been regarded as a scapegoat for explaining changes in the increased vascular permeability in the course of diabetic retinopathy. However, the blood-retinal barrier function of the endothelial cells is supported by surrounding cells, such as Müller cells, pericytes, and astrocytes [3]. Since the blood-retinal barrier depends so heavily on this interdependent microenvironment where the function of one cell type depends on support from other cell types, any cellular injury and cell loss will have vast effects on proper retinal barrier function and for that matter any retinal function [4-6].

Indeed, loss of retinal cells seems to be a prominent feature of diabetic retinopathy. Diabetes-induced cell death has been observed in numerous retinal cell types such as endothelial cells [5,7-9], pericytes [9-11], neural retinal cells such as ganglion cells [12-14], and retinal glial cells such as Müller cells, astrocytes, and microglia [15-23]. Endothelial cell death and pericyte loss have long been assumed to play an important role in the loss of proper blood-retinal barrier function [4,9,24,25]. Despite increasing efforts to demonstrate retinal cell death in diabetic retinopathy, mechanisms leading to cell death by diabetes are only poorly understood to date. Identifying potential modes of cell death is complicated by the fact that for some forms of cell death, the pathways and markers are poorly understood and are still being discovered. In recent years, existing types of cell death (apoptosis and necrosis) have been re-classified and new subtypes of cell death have been added.

According to the most recent cell death nomenclature paper published by the Nomenclature Committee on Cell Death (NCCD) there are now 13 subroutines of regulated cell death identified [26]. These include anoikis, autophagic cell death, caspase-dependent intrinsic apoptosis, caspase-independent intrinsic apoptosis, cornification, entosis, extrinisic apoptosis by death receptors, extrinsic apoptosis by dependence receptors, mitotic catastrophe, regulated necrosis, netosis, parthanatosis, and pyroptosis. Each type of cell death has different, and often not fully defined, characteristics and markers leading to increased complexity in correct identification of cell death mechanisms both *in vitro* and more importantly *in vivo*. Apoptosis is the most studied type of cell death in diabetic retinopathy. It has well-defined features and is easily detectable with established techniques, such as TUNEL (Terminal dUTP Nick End Labeling) assay. However, some of the cell death types are far more difficult to detect due to the lack of established markers and techniques available.

In order to establish mechanisms underlying the development of diabetic retinopathy and to determine whether cell death is crucial for the progression of the disease, a better understanding of potential types of cell death in the diabetic retina must be achieved. This will then allow for more targeted therapies to combat cell death in diabetic retinopathy. This review will provide an overview of the various retinal cell types undergoing cell death in diabetic retinopathy and attempt to assign cell death classification to these dying cells.

Apoptosis: Extrinsic Versus Intrinsic

The most well defined form of cell death is apoptosis. Apoptosis, originally introduced by Kerr et al. in 1972, is a term that describes a form of programmed cell death resulting in cytoplasmic and nuclear

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condensation, a specific pattern of DNA fragmentation, and eventual demise of the cell into apoptotic bodies to be phagocytosed by surrounding cells with very little inflammation involved in the process [27]. The common theme in identification of apoptotic cell death is the use of TUNEL staining or other methods that are aimed to specifically detect the apoptotic DNA laddering pattern [28-30]. However, due to recent advancements in cell death studies and changes in cell death nomenclature, classification of apoptosis is not as simple as detection of specific DNA fragmentation.

Apoptosis can be divided into various subcategories according to both the stimuli and the pathways leading to execution of cell death, and should therefore be a term used with caution. 'Extrinisic Apoptosis' for example is used to define cell death induced by binding of lethal ligands including FAS/CD95 ligand, tumor necrosis factor α (TNFα), or TNF-related apoptosis inducing ligand (TRAIL) to their respective death receptor [26,31]. Upon binding of these ligands to the death receptor, the "death domain" of the receptor recruits the assembly of the "death-inducing signaling complex" (DISC), a platform of various proteins. The DISC can differ depending on the death receptor involved but typically results in activation of caspase-8 [26,32-34]. Depending on cell type, active caspase-8 initiates one of two distinct pathways. First, active caspase-8 can directly cleave caspase-3, known as an executioner caspase in the apoptotic process [35]. Alternatively, active caspase-8 can cleave BH3-interacting domain death agonist (BID) creating truncated BID (tBID). tBID then binds Bcl-2 allowing BAX to form a pore in the outer membrane of the mitochondria enabling the release of cytochrome c into the cytosol. This triggers formation of the canonical 'apoptosome' via assembly of APAF1 with pro-caspase-9, cytochrome c, and dATP leading to caspase-9 activation, which in turn activates caspase-3 [26,36].

Another type of apoptosis, 'intrinsic apoptosis' is similar to extrinsic apoptosis in that there is eventual activation of caspase-3 as the executioner caspase. However, rather than an extrinsic ligand binding to a death receptor, apoptosis is triggered by intracellular stress such as DNA damage, oxidative stress, or excitotoxicity [26]. Regardless of the intracellular stress that initiates intrinsic apoptosis, the intrinsic and extrinsic pathways converge at the mitochondria. Increased pore formation by either bak or bax, or pore formation by a multi-protein complex termed the permeability transition pore (PTP) promotes the release of proteins such as cytochrome c, apoptosis-inducing factor (AIF), and endonuclease G (ENDOG) from the mitochondria into the cytosol [26,37,38]. In addition, alterations of the respiratory chain lead to increased reactive oxygen species (ROS) production [26]. As described above, apoptosome formation induces caspase-9 and subsequent caspase-3 activation. Activation of caspse-3 initiates events that are responsible for the specific DNA cleavage pattern seen in apoptotic cell death.

In contrast, AIF and ENDOG can translocate to the nucleus leading to DNA fragmentation that is independent of caspase activation [36,39-44]. In this case, apoptotic cell death occurs even in the absence of active caspases or when caspases are pharmacologically inhibited. This allows for even further classification of intrinsic apoptosis into caspase-dependent and caspase-independent intrinsic apoptosis [26]. Therefore, observation of DNA fragmentation alone by TUNEL staining is not sufficient to distinguish between the different types of apoptosis.

Much of the research in diabetes-induced retinal cell death has been focused on identifying apoptosis using the TUNEL assay as the method of choice. Some TUNEL based studies were supported by additional data identifying active caspase-3. TUNEL staining has identified increased endothelial cell apoptosis in retinas of diabetic and galactose fed rats, compared to control animals [9,45,46]. A similar increase in TUNEL staining was seen in retinal endothelial cells of diabetic mice [47]. A recent study confirmed these results in the retinas of human subjects with diabetic retinopathy compared to those without [48]. TUNEL staining has also been used in studies showing that neutrophils from diabetic rats, when co-cultured with human endothelial cells, led to increased endothelial cell apoptosis indicating that other cells types when exposed to hyperglycemia induce endothelial cell death via an apoptotic pathway [49]. In other studies, propidium iodide (PI), which when injected intravenously will fluoresce after leakage through injured cell membrane and bind to DNA or RNA, has been used to detect endothelial cell apoptosis in diabetic rats [7]. However, PI staining does not allow for discrimination between cells undergoing apoptosis or necrosis [7]. While much of the apoptosis research has used DNA fragmentation alone, some more detailed studies have shown that high glucose leads to cytochrome c release and changes in mitochondrial morphology in endothelial cells indicating a mitochondria-mediated apoptotic mechanism [50]. This is further supported by a study that demonstrated that overexpression of bcl2, an anti-apoptotic member of the bcl2 family, prevented capillary degeneration in diabetic mice [51]. Other studies have demonstrated that hyperglycemia can initiate pro-apoptotic pathways in endothelial cells by measuring caspase-8 and caspase-3 activity indicating the caspase dependency of the apoptotic process [5,47,52,53]. Although these studies provide good evidence that caspase-dependent apoptosis is the predominant type of cell death for endothelial cells when exposed to a hyperglycemic environment, more studies are needed to determine whether apoptotic cell death in endothelial cells is mediated by an intrinsic or extrinsic mechanism in diabetic retinopathy.

Apoptosis has also been suggested as the type of cell death in pericytes during the progression of diabetic retinopathy. TUNEL staining demonstrated increased pericyte apoptosis in retinas of diabetic and galactose fed rodents compared to control animals [9,45,54]. Increased pericyte apoptosis has also been shown in retinal tissue of diabetic patients compared to non-diabetic patients, again using TUNEL staining [10,11]. In addition, increased caspase-8 and caspase-3 activity is seen in rat retinal pericytes in high glucose conditions [55]. Similarly to endothelial cells, mitochondria of retinal pericytes display significant fragmentation and metabolic dysregulation and this has been directly implicated in accelerated apoptosis in retinal pericytes in diabetic retinopathy [56]. These studies all indicate a similar caspase-dependent apoptotic mechanism for pericytes as seen for endothelial cells.

Additionally, TUNEL staining has been used to identify apoptosis in a variety of retinal cell types, although numbers of these studies are limited. Increased apoptosis in neural retinal cells such as ganglion cells of diabetic rats compared to non-diabetic rats has been detected [12,13,18,46,54]. Amacrine cells have also been shown to undergoapoptosis, as characterized by TUNEL staining and staining for active caspase-3, using the Ins2Akita mouse model [57]. It has been suggested that there is selective S-cone loss as identified by TUNEL staining in diabetic retinopathy [58]. However, more detailed studies are needed to further confirm the aforementioned mechanisms and to allow for a distinct classification of apoptosis in these cells. Although numerous studies have established that apoptosis of several cell types occurs in the diabetic retina, the next important step will be to identify the link between increased glucose levels and the initiation of caspase-dependent apoptosis.

Necrosis: A Regulated Pathway to Cell Death?

Historically, necrosis was considered the type of cell death "on the other end" of the cell death spectrum. Necrosis has been a term used for 'accidental cell death' rather than 'programmed cell death,' which was reserved for apoptosis. It was defined in the classification of cell death article published by the NCCD in 2005, as cell death with no apparent signs of apoptosis or autophagy [59]. The morphological appearance of cells undergoing necrosis were described as having features such as cytoplasmic swelling, mechanical rupture of the plasma membrane, dilation of cytoplasmic organelles, and chromatin condensation [59]. The understanding of pathways leading to necrosis *in vivo* was vague at best. Due to the lack of a clear mechanism for necrosis, new terms describing "necrosis-like" cell death were introduced. One of these new terms was "apoptonecrosis" where apoptosis evolves into necrosis, although use of this term was discouraged to avoid further confusion until pathways involved in this process were fully identified [59]. However, out of this research, the picture of "regulated necrosis" and it's importance in various physiological and pathological settings evolved [26,60]. Triggers for regulated necrosis include excitotoxicity, DNA damage resulting in DNA alkylation, and ligands such as TNF and FasL binding to their respective death receptors [26,61-65]. These triggers initiate ubiquitination of receptor interacting kinase (RIP) 1 and subsequent activation of RIP3. Whereas RIP3 would activate pro-caspase-8 in apoptotic conditions, in experimental or pathological settings where caspase-8 is absent RIP3 can lead directly to execution of regulated necrosis [26,61-63]. Crucial characteristics of regulated necrosis include death receptor signaling, absence of caspase activity, and RIP1 and /or RIP3 activation. Activation of pathways in regulated necrosis still lead to the classical morphological features associated with necrosis [26]. All these new studies indicate that the process of necrotic cell death can be regulated depending on microenvironment rather than being a random event as previously assumed.

Necrosis has been implicated in the process of diabetic retinopathy. Increased necrotic cell death of pericytes has been observed in the retinas of diabetic rats and humans using light and electron microscopy [66-68]. This particular pericyte cell death was later described as "selective necrosis" [69]. Reasoning for this designation was most likely due to the assumption that this cell death caused by diabetic conditions was accidental. Although the newer studies claim apoptosis as the major type of cell death for pericytes in diabetic retinopathy, one cannot exclude that some pericytes might undergo cell death via regulated necrosis depending on microenvironment and the progression of the disease. Further clarification of the definition for necrosis and the pathways involved may be necessary to better understand and identify this process in the diabetic retina.

Autophagic Cell Death

Autophagic cell death may be the most puzzling type of cell death identified to date. It is currently defined by the NCCD as "a type of cell death that occurs in the absence of chromatin condensation but accompanied by massive autophagic vacuolization of the cytoplasm" [70]. The first study demonstrating that autophagic cell death exists *in vivo* showed that knockdown of key genes required for autophagy reduced cell death in *Drosophila melanogaster* [71]. Autophagic cell death has also been identified in cancer cells exposed to chemotherapeutic agents *in vitro* [72,73]. Cells dying by autophagic cell death have very little association with phagocytes, contrary to cells dying by apoptosis which are eventually removed via phagocytosis [70]. In order to determine autophagic cell death, cell death must be prevented by inhibition of the autophagic pathway either by chemicals or knockdown of essential autophagic proteins [26,70]. Detection of common markers used to observe increases or decreases in autophagy, such as LC3 (microtubule-associated protein 1 light chain 3) or ATG (autophagy) family members, are not sufficient to indicate autophagic cell death.

Autophagy is the process of removing unwanted or damaged cellular material or organelles by packaging these materials into autophagosomes, which are then targeted for degradation. In most physiological settings, autophagy is considered a beneficial and a pro-survival mechanism used by the cell and inhibition of autophagy can actually lead to increased apoptosis [74-78]. Therefore, an increase in autophagic flux does not always imply autophagic cell death. For example, if cell death occurs with increased markers of autophagy but cannot be blocked by autophagy inhibition, it is not indicative of autophagic cell death. To classify cells as dying by autophagic cell death, inhibition of proteins within the autophagic pathway must promote cell survival.

Whether autophagic cell death is occurring in the progression of diabetic retinopathy has not been determined to date. A recent study indicated increased autophagy by measuring levels of ATG5, but whether this ultimately leads to autophagic cell death in retinal cells during diabetic retinopathy has not been addressed [79].

Pyroptosis: Inflammation Driven Cell Death

An emerging type of cell death that is attracting increasing attention is 'pyroptosis.' Pyroptosis is an inherently inflammatory-mediated form of cell death, defined as being caspase-1-dependent [26,80,81]. During pyroptosis, there is assembly of a multiprotein platform allowing for induced proximity-mediated activation of caspase-1. Active caspase-1 then cleaves the pro-inflammatory cytokines IL-1β and IL-18 from their inactive precursors to their biologically active forms [80-83]. The multiprotein platform allowing for caspase-1 activation is termed either the inflammasome or pyroptosome. Inflammasomes are comprised of the ASC (Apoptosis-associated Specklike protein containing a CARD) adaptor protein and a cytosolic sensor of either DAMPS (Danger Associated Molecular Patterns) or PAMPS (Pathogen Associated Molecular Patterns) such as a NLRs (NOD-like receptors) or AIM2 (Absent In Melanoma 2) [84-88]. The pyroptosome is an assembly of ASC dimers that can directly activate caspase-1 [89]. Prevention of pyroptosis is accomplished by inhibition of caspase-1 either by pharmacological intervention or caspase-1 knockout in animal models. Although it is now very well established that initiation of pyroptosis is caspase-1 and IL-1β driven, the execution phase of pyroptosis is not yet completely understood. It has been shown that pyroptosis shares traits with both apoptosis and necrosis in the execution phase [70,90]. Execution of pyroptotic cell death might depend on cell type, microenvironment, and stimulus. Different pathways might be involved in the execution of pyroptosis bringing into question whether TUNEL staining is actually able to identify all pyroptotic cells.

This inflammatory-mediated process of cell death is particularly intriguing in the context of diabetic retinopathy. A disease which was originally thought of as a purely microvascular disease, diabetic retinopathy is now being viewed as a potential chronic inflammatory disease leading to changes in the retinal microvasculature [1,91,92]. Studies have demonstrated that diabetes leads to activation of caspase-1 and IL-1β production in the retinas of diabetic and galactosemic mice as well as diabetic rats [18,93,94]. Active caspase-1 and IL-1β was also detected in retinal tissue of diabetic patients [15,16] and the vitreous

of patients with proliferative diabetic retinopathy [95,96]. Inhibition of the caspaspe-1/IL-β signaling pathway prevented the development of diabetic retinopathy in diabetic and galactosemic animals indicating that this inflammatory pathway is important for disease development, potentially via pyroptotic cell death of retinal cells that are crucial for proper retinal function [16,18].

When looking at specific cell types undergoing pyroptotic cell death in the course of diabetic retinopathy, retinal glial cells stand out. It has been shown that caspase-1 activity and IL-1β production is increased *in vitro* in Müller cells following exposure to hyperglycemic conditions and cells die as a consequence [97,98]. Inhibition of the caspase-1 pathway prevented Müller cell death under these conditions. In Müller cells and microglia, it has been shown that use of minocycline, a drug that decreases caspase-1/IL-1β signaling, is able to prevent cell death [16,18]. These *in vitro* studies are an indication that glial cells might respond to chronically elevated glucose levels by undergoing pyroptotic cell death.

Since execution of pyropototic cell death lacks specific markers, identifying retinal cells dying by pyroptosis *in vivo* is a difficult task. Studies using EM show that there is Müller cell death occurring in diabetic retinopathy [69]. Dying Müller cells are described as being hypertrophic consistent with the notion that during pyroptosis, cells swell rather than shrink as observed in apoptotic cell death [99]. Other studies indicate that there is simply hypertrophy and glial dysfunction associated with the disease [18]. A previous study by us has shown that GAPDH (glyceraldehyde-3-phosphate dehydrogenase) accumulates in the nucleus of Müller cells in the retinas of diabetic rats [17]. Nuclear accumulation of GAPDH has been closely associated with cell death induction [100-102]. Interestingly, hyperglycemia-induced nuclear accumulation of GAPDH was mediated by activation of the caspase-1/IL-1β pathway [103,104]. Whether caspase-1/IL-1β-mediated GAPDH nuclear accumulation is part of the pyroptotic pathway in general has yet to be determined. As our results indicate (Figure 1), diabetes leads to Müller cell loss in the retinas of diabetic mice. Due to the lack of specific markers for pyroptotic cell death, Müller cells were stained against glutamine synthetase and CRALBP (cellular retinaldehyde-binding protein) and counted. To confirm that Müller cell loss was dependent on the activation of the caspase-1/IL-1β pathway, IL-1 receptor knockout mice were made diabetic and Müller cells were counted in retinas of non-diabetic and diabetic IL-1 receptor knockout mice. Inhibition of the caspase-1/IL-1β pathway prevented diabetes-induced Müller cell loss. These studies are the first to clearly demonstrate Müller cell loss in diabetes and to suggest that cell death might occur via a pyroptotic mechanism. Based on our studies, we suggest that hyperglycemia leads to activation of caspase-1 and subsequent production of IL-1β leading to Müller cells death via pyroptosis (Figure 2). Since glial cells in general respond to hyperglycemia by producing pro-inflammatory cytokines, future studies need to determine whether all glial cell types are able to undergo pyroptotic cell death.

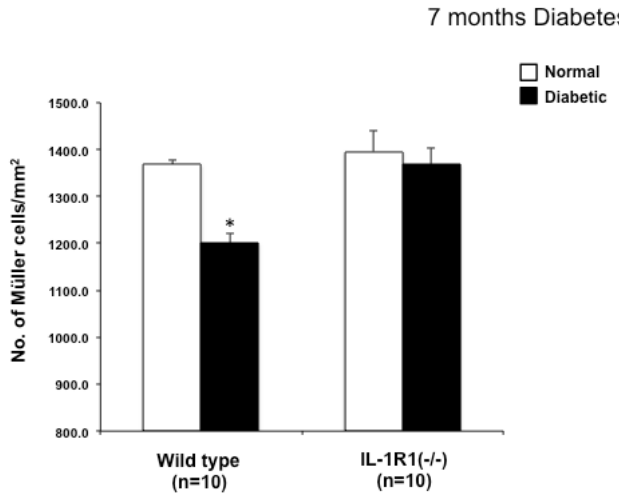

Figure 1: Diabetic wild type (gHb= 11.0 ± 1.8) and IL-1R1$^{-/-}$ (gHb= 12.1 ± 0.4) were sacrificed after 7 months of diabetes along with age matched normal controls (wild type gHb= 3.8 ± 0.55, IL-1R1$^{-/-}$ gHb= 3.2 ± 0.2). Animals were scarified and eyes were isolated and fixed in formalin. Retinal sections were processed for both glutamine synthase, CRALBP, and DAPI staining and blinded samples were visualized using confocal microscopy Z-sections. The number of Müller cells was determined by counting three independent areas per retinal section and three retinal sections per animal. The number of Müller cells per mm^2 retina is expressed as mean ± STDV (n=10 per group).

Conclusion

In conclusion, cell death seems to be a prominent feature in the progression of diabetic retinopathy. Several retinal cell types have been shown to undergo various forms of cell death (Table 1). A lot of emphasis has been given to study apoptosis of retinal cells during the progression of the disease. Based on the studies available, it is fair to say that endothelial cells are predominantly dying by an apoptotic process, however the type of apoptosis has yet to be determined. Several studies point to a caspase-dependent mechanism involving mitochondrial damage but more studies are needed to fully determine an intrinsic or extrinsic apoptotic pathway. Other cell types suggested to undergo apoptosis are ganglion cells, amacrine cells, and S-cones. However, due to the limited number of studies on cell death in these cell types, a conclusion as to whether these cells are truly dying of apoptosis cannot be made at this time. The picture is not clear for pericytes; both apoptosis as well as necrosis have been suggested as modes of cell death. Given the new research on necrosis that indicates necrosis can also be a regulated process like apoptosis, more studies are needed to determine precise mechanisms of pericyte cell death in diabetic retinopathy. The type of cell death might depend on the phenotype of pericytes and the microenvironment surrounding these cells. Inflammation-driven pyroptosis is an emerging form of cell death that is receiving a lot of attention right now. This type of cell death is intriguing to study in the context of diabetic retinopathy since new understandings of the disease suggest that diabetic retinopathy is a chronic inflammatory disease. Müller cells are prime candidates for this form of cell death due to the fact that diabetes-induced cell death is dependent on the

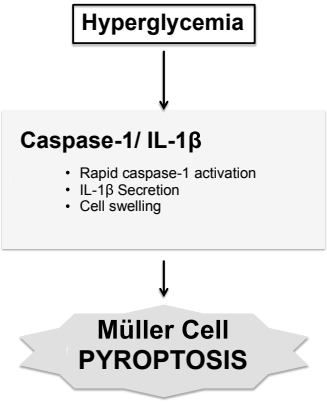

Figure 2: Scheme of pyroptosis in Müller cells.

	Cell Death Characteristics	Retinal Cell Types
Extrinsic Apoptosis	• Triggered by binding of lethal ligands (FAS/CD95, TNF, TRAIL) to respective death receptor • Caspase-8 activation leading to eventual caspase-9 and caspase-3 activation as the executioner caspase • DNA fragmentation	• Endothelial cells • Pericyte • Ganglion cells • Amacrine • S-cones
Intrinsic Apoptosis	• Triggered by intracellular stress (DNA damage, oxidative stress, excitoxicity) • Increased mitochondrial outer membrane permeability • Release of proteins from mitochondria (cytochrome c) • AIF and ENDOG nuclear translocation • DNA fragmentation • Caspase-9 activation leading to caspase-3 activation as the executioner caspase	• Endothelial cells • Pericytes • Ganglion cells • Amacrine • S-cones
Necrosis	• Triggered by excitotoxicity, DNA damage, or binding of lethal ligands (TNF, FasL) • Ubiquitination of RIP1 and subsequent RIP3 activation • Cell death even in the absence of caspase activity	• Pericytes
Autophagic cell death	• Presence of autophagy markers (lipidation of LC3/Atg8) • Increased degradation of autophagic substrates (SQSTM1) • Prevented by inhibition of autophagy	• ?
Pyroptosis	• Triggered by either DAMPs or PAMPs • Assembly of either inflammasome or pyroptosome complex • Caspase-1 activation and subsequent IL-1β or IL-18	• Müller cells

Abbreviations: AIF: Apoptosis-inducing Factor; ENDOG: Endonuclease G; TNF: Tumor Necrosis Factor; LC3/Atg8: Microtubule-associated protein 1 light chain 3; SQSTM1: Sequestosome 1; DAMP: Danger Associated Molecular Pattern; PAMP: Pathogen Associated Molecular Pattern

Table 1: Characteristics of modes of cell death and potential retinal cell types undergoing cell death in diabetic retinopathy (for more detailed list of characteristics of cell death modes refer to current NCCD definitions [26]). Retinal cell types were assigned into a cell death category based on identification of at least two characteristics of the respective mode of cell death.

activation of the caspase-1/IL-1β pathway. More studies are needed to fully understand the mechanism underlying the process of pyroptosis and to determine whether glial cells including macro and micro glial cells are undergoing pyroptosis in diabetes. Loss of other retinal cell types such as astrocytes has been reported but identification of the type of cell death has not been made [23].

Other types of cell death such as anoikis, entosis, parthanatos, netosis, cornification, and mitotic catastrophe, have yet to be identified in the course of diabetic retinopathy but cannot be excluded from the process of disease development. Further development of tools capable of assessing these modes of cell death is needed to determine whether these cell death modalities are present in diabetic retinopathy.

A better understanding of how retinal cells are dying during the development and progression of diabetic retinopathy will allow for a more targeted approach to intervene in this process. Although the general consensus is that inhibition of cell death is beneficial for disease prognosis, timing at which intervention should be started and targeting of specific retinal cell types will be crucial for a successful outcome of treatments aiming to inhibit cell death. Therefore, the more knowledge that is attained on which cell types undergo what type of cell death, the more therapeutic strategies can be developed.

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