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Ceramide Signaling in Retinal Degeneration

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Abstract

Retinal degenerations (RD) are a complex heterogeneous group of diseases in which retinal photoreceptors and the supporting retinal pigment epithelial cells die irreversibly, causing visual loss for millions of people. Mutations on more than 150 genes have been discovered for RD and there are many forms that possess complex etiology involving more than one gene and environmental effect. For years many have searched for some common intracellular second messenger for these many forms of cell death which could be targeted for therapy. Ceramide is a novel cellular second messenger which signals for apoptosis. Several lines of evidence suggest an integral role of ceramide in photoreceptor apoptosis and cell death. Understanding their role in the pathogenic pathways of retinal degenerative diseases is important for development of targeted therapeutics.

70.1 Introduction

Ceramide (Cer) is the key metabolite of cellular sphingolipids, which are a family of membrane lipids with important structural roles in the regulation of the fluidity and subdomain structure of the lipid bilayer, especially lipid rafts (Tsui-Pierchala et al. 2002; Martin et al. 2005); it also has crucial functional roles in receptor function, in membrane conductance, in cell–cell interactions, and in the internalization of pathogens (Huwiler et al. 2000; Hannun and Obeid 2008). But their role in signaling, since the discovery of Cer as a mediator of apoptotic cell death, received wider attention in the last two decades (Obeid et al. 1993; Hannun and Obeid 2008). It is now an established fact that bioactive sphingolipids, such as Cer, ceramide-1-phosphate (C1P), sphingosine (Sph), and sphingosine-1-phosphate (S1P), maintain cellular homeostasis by regulating cell growth, apoptosis, inflammation, angiogenesis, and neovascularization (Olivera and Spiegel 1993; Spiegel and Milstien 2003; Futerman and Hannun 2004; Hannun and Obeid 2008). The processes of apoptotic cell death is integral to major retinal diseases including Retinitis Pigmentosa (RP), Stargardt's disease, Leber's congenital amaurosis (LCA), and age-related macular degeneration (AMD) (Glazer and Dryja 2002; Allikmets 2004; Haddad et al. 2006; Hartong et al. 2006). Recent evidence suggests a strong correlation between Cer signaling and survival and homeostasis of photoreceptor and retinal pigment epithelial (RPE) cells, which is the focus of this review.

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70.2 An Overview of Ceramide Metabolism in the Cell

There are two major pathways that exist for Cer production in the cell. The first is the *de novo* biosynthesis of ceramide, which takes place in the endoplasmic reticulum (ER) through condensation of L-serine and palmitoyl-CoA by the enzyme serine-palmitoyl transferase (SPT) followed by sequential generation of 3-ketodihydrosphingosine (3-keto-DHS), dihydrosphingosine (DHS), and dihydroceramide, before finally being converted into Cer. The second pathway for cellular Cer generation is from the hydrolysis of higher-order sphingolipids, mainly sphingomyelin (SM), in the plasma membrane by the action of sphingomyelinase (SMase, or Sphingomyelin phosphodiesterase, SMPD) enzymes. The catabolic pathway of ceramide degradation is catalyzed by a group of enzymes called ceramidases (or ASAH, acyl-sphingosine-amido-hydrolase), which deacetylate Cer to yield Sph and a fatty acid. Both Cer and Sph can be phosphorylated by specific kinases to form C1P and S1P, respectively.

70.3 Ceramide in Photoreceptor Apoptosis

Photoreceptor death by apoptosis is the hallmark of most retinal degenerative disorders (Chang et al. 1993; Portera-Cailliau et al. 1994; Carella 2003). Recently, from *in vitro* studies, Cer has been established as an essential second messenger in the activation of apoptosis in photoreceptors. In rat retina neuronal cultures, Nora Rotstein's group showed that addition of cell permeable short-chain C_2-Cer triggers photoreceptor apoptosis, whereas inhibition of synthesis of endogenous Cer protects photoreceptors from oxidative stress-induced (paraquat) apoptosis (German et al. 2006). In a mouse retina-derived 661W cell line, treatment with sodium nitroprusside showed an increase in Cer levels, while inhibition of this increased Cer by desipramine, an inhibitor of SMase enzymes, protected these cells from oxidative stress-induced apoptosis, suggesting a role of Cer in oxidative stress induced photoreceptor cell death (Sanvicens and Cotter 2006). In our lab, we tested cell-permeable C_8-Cer and BSA-conjugated C_{16}-Cer (physiological Cer) in 661W cells, and found both these ceramides caused cell death in a dose dependent manner; we also observed an increase in Cer H_2O_2 treated 661W cells (Mandal et al. unpublished data), providing additional support of Cer's role as a death mediator in retinal cells. In another recent publication, Rotstein's group showed that enhanced formation of Cer and its subsequent breakdown to Sph, which was induced by oxidative stress, triggered photoreceptor apoptosis in cultured rat retina neurons (Abrahan et al. 2010). In summary, these evidences point toward a key role for Cer as a common mediator of photoreceptor cell apoptosis in culture, especially due to oxidative stress.

Evidence is gradually accumulating on the role of Cer in photoreceptor cell death *in vivo,* too. The first evidence came from *Drosophila* studies, in which Acharya et al. detected increased Cer in arrestin2 (*arr2*) and phospholipase C (*plc*) mutant photoreceptors. Further, transgenic over-expression of neutral Ceramidase (nCDase or ASAH2), an enzyme that reduces Cer level by breaking Cer into Sph and free fatty acid, showed prevention of photoreceptors from Cer-induced cell death (Acharya et al. 2003; Acharya et al. 2004). They also showed that preventing *de novo* biosynthesis of Cer suppresses retinal degeneration in *Drosophila* phototransduction mutants. Noteworthy, over-expression of ceramidase in tissues distant from photoreceptors suppresses photoreceptor degeneration in an arrestin mutant and facilitates membrane turnover in a rhodopsin null mutant (Acharya et al. 2008). Mutation in ceramide kinase gene in *Drosophila* leads to photoreceptor degeneration, also by accumulation of excess Cer, and provides additional evidence of Cer involvement in photoreceptor cell death (Dasgupta et al. 2009). In mutants, the accumulated Cer subsequently leads to loss of phospholipase C activity and inhibits phototransduction, which is then accompanied by severe degeneration of photoreceptors. Over expression of

ceramidase in these cells decreased Cer levels and rescued PLC activity and cell death (Dasgupta et al. 2009).

Further, in a mammalian model (rabbit), involvement of Cer has been associated with a gradual loss of photoreceptor cells in an experimental model of retinal detachment (Ranty et al. 2009). Using light-damaged albino rat models and with genetic models of retinal dystrophies (RCS and P23H1 rats), we found an integral association of increased Cer level with photoreceptor cell death (Mandal et al. unpublished data). A recent publication shows that ceramide levels increase with progression of photoreceptor degeneration in *Rd10* mice and that inhibiting *de novo* ceramide biosynthesis by myriocin (a powerful inhibitor of SPT enzyme) can preserve photoreceptor structure and function in this mouse model of RP (Strettoi et al. 2010a).

In human, mutations in *Ceramide kinase like* (*CERKL*) gene are found to be associated with inherited RP (RP26) (Tuson et al. 2004; Auslender et al. 2007). The function of CERKL is not yet known but is potentially involved in the Cer metabolic pathway, suggesting a direct link between human retinal degeneration and Cer-mediated apoptosis. Additionally, in several inherited sphingolipid metabolism defect diseases commonly known as ‘lipid storage disease’, such as Krabbe’s disease (Brownstein et al. 1978), Niemann-Pick disease (Robb and Kuwabara 1973), Sandhoff disease (Sango et al. 2008), and Gaucher disease (Seidova et al. 2009), retinal impairment and vision loss due to retinal neuronal cell death are often present and accompanied by Cer accumulation. Increased level of Cer was found in retinas of patients with Farber disease, caused by a mutation in *Ceramidase* gene (also known as ceramidase deficiency) (Zarbin et al. 1985; Zarbin et al. 1988). Furthermore, increased Cer was found in the brain of patients with the juvenile form of Batten disease (Puranam et al. 1997), in which neuronal apoptosis is thought to be the cause of RP in these patients. Cer is also shown to alter the chloride channel activity of Bestrophin protein, and Cer accumulation is suggested to enhance inflammation in the retina in Best vitelliform macular dystrophy (VMD) patients (Xiao et al. 2009). In summary, the changes in Cer level in human and mouse inherited retinal diseases, proof of Cer involvement in *Drosophila* photoreceptor degeneration, involvement of Cer in cell death in vitro, and the association between Cer changes and retinal neuronal death in the sphingolipid metabolic diseases underscore the significance of Cer and its metabolites in the death of retina photoreceptors and retina degeneration diseases.

70.4 Ceramide Signaling in RPE Cell Death

RPE atrophy (cell death) followed by or concomitant with photoreceptor cell death is the final outcome of all forms of RP. In dry AMD, the atrophy to the RPE cell in the macular region is considered to be the primary pathology, and is also common in the early stage of wet AMD. Recent evidences have shown that Cer participates in activating RPE cell death. In cultured human RPE (hRPE) cells, treatment with the chemical oxidants tBH and H_2O_2 led to Cer generation, which signaled for RPE apoptosis (Barak et al. 2001). In another study, laser exposure induced hRPE apoptosis with concomitant Cer production (Barak et al. 2005). Further studies on the over-expression of Sphingomyelin phosphodiesterase-3 (SMPD3), a key enzyme responsible for Cer production from SM, showed enhanced RPE cell death and arrested cell proliferation, with the percentage of apoptotic cells increasing proportionally with the amount of transfected *SMPD3* DNA (Zhu et al. 2010). Interestingly, the short-chain C_2-Cer selectively induced apoptosis in the non-polarized RPE cultures but not in fully differentiated and polarized RPE cells. This has a relevance with AMD since non-polarized RPE cells are found in late age-related macular degeneration lesions, and Cer may play a critical role in their apoptosis (Zhu et al. 2010). The studies on the mechanisms of Cer-induced RPE apoptosis indicated the role of increasing ROS production,

mitochondrial membrane permeability transition (MPT), and caspase-3 activation (Kannan et al. 2004).

70.5 Conclusion

Cer is known as a deadly second messenger in the cell and is now known to play a role in various forms of photoreceptor cell death. This discovery has a significant impact from a therapeutic point of view; Cer synthesis can be targeted for retinal degenerative diseases and evidence has already started accumulating from *Drosophila* and mammalian studies (Acharya et al. 2003; Strettoi et al. 2010a). Cer metabolism is complex and other bioactive Cer metabolites, such as C1P and S1P, also play important roles in cell growth, differentiation, inflammation and neovascularization, which are relevant in many forms of retinal degenerative diseases, recently reviewed in Rotstein et al. 2010 and. which is beyond the scope of this review.

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