cAMP/PKA pathway and mitochondrial protection in oxidative stress-induced optic nerve head astrocytes

Keun-Young Kim, Won-Kyu Ju*

Oxidative stress and cyclic adenosine 3′,5′-monophosphate (cAMP)/protein kinase A (PKA) pathway in the optic nerve head astrocytes: Glaucoma is a leading cause of blindness worldwide in individuals 60 years of age and older. Despite the widely appreciated disease relevance of structural and functional abnormalities of astrocyte in the optic nerve head (ONH) that is associated with retinal ganglion cell (RGC) axon degeneration, the molecular mechanisms underlying astrocyte dysfunction in glaucomatous ONH degeneration are poorly understood. Oxidative stress is strongly linked to glaucoma pathogenesis, and astrocytes are the responsible cell type that is mostly related to oxidative stress and glaucomatous ONH degeneration.

Accumulating evidence indicates that increased levels of cAMP are associated with ONH astrocyte alteration from patients with primary open-angle glaucoma (POAG) and experimental rodent glaucoma (Lukas et al., 2008; Shim et al., 2017, 2018). The ubiquitous second messenger cAMP in the central nervous system contributes to numerous biological processes, including cell growth and death. Upon stimulation, cAMP synthesis and its degradation are tightly regulated by adenylyl cyclases and cyclic nucleotide phosphodiesterases, respectively.

Our recent study provides evidence that glaucomatous ONH astrocytes in the glial lamina of aged DBA/2J mice increase the expression levels of cAMP as well as Bax and caspase-3 proteins (Shim et al., 2018). In parallel, glaucomatous ONH astrocytes showed morphological alterations such as hypertrophic cell bodies and retracted processes, as well as a loosen arrangement or loss of processes in the normal condition (Ju et al., 2015; Shim et al., 2018). These results importantly suggest the possibility that increasing the cAMP level is associated with ONH astrocyte dysfunction or death in glaucoma pathogenesis.

More importantly, we have demonstrated for the first time that the elevated intracellular cAMP/PKA signaling exacerbates vulnerability to oxidative stress in ONH astrocytes (Shim et al., 2018). In this study, itnhas been shown that the activation of the cAMP/PKA signaling pathway significantly reduces the level of AKT phosphorylation at serine 473, which is involved in glial protection against oxidative stress. Furthermore, the inhibition of cAMP/PKA pathway activation protected ONH astrocyte death against oxidative stress via increasing AKT phosphorylation at serine 473 and blocking Bim/Bax pathway and caspase-3 activation. Based on these findings, we propose the notion that the elevated cAMP-mediated PKA pathway plays a critical role in oxidative stress-mediated astrocyte dysfunction in glaucomatous ONH degeneration.

Astrocytes play essential roles in the maintenance of unmyelinated axons and energy support to the axons in the ONH (Li et al., 2015). In this aspect, our results suggest that oxidative stress-induced dysfunctional astrocytes may not only trigger energy deficiency to axons but also accelerate axonal degeneration during glaucomatous ONH degeneration. However, the relationship between the cAMP/PKA pathway and mitochondrial dysfunction in oxidative stress-induced ONH astrocytes remains unknown. In this perspective article, we will address our recent evidence that highlights the importance of cAMP/PKA pathway on mitochondrial dysfunction and protection in glaucomatous ONH astrocytes.

Oxidative stress and mitochondrial dysfunction in optic nerve head astrocytes: POAG is strongly associated with 1) polymorphism of mitochondrial cytochrome c oxidase subunit I of the oxidative phosphorylation (OXPHOS) complex (Cx) IV and 2) impaired OXPHOS Cx-I-linked respiration activity and adenosine triphosphate (ATP) synthesis (Collins et al., 2018), suggesting that compromised OXPHOS mediates mitochondrial dysfunction in glaucoma pathogenesis. However, the molecular mechanisms underlying OXPHOS stress and how impaired mitochondrial network and bioenergetics contribute to glaucoma remain obscure. In particular, evidence from our group strongly indicates that mitochondrial dysfunction and metabolic stress by glaucomatous insults such as elevated intraocular pressure, glutamate excitotoxicity and oxidative stress are critical to not only loss of RGCs but also dysfunction of ONH astrocytes in experimental glaucoma (Ju et al., 2015; Kim et al., 2015; Shim et al., 2018). These findings point to a strong link between OXPHOS-mediated mitochondrial dysfunction and glaucoma.

Recent evidence indicates that vascular abnormalities induced by elevated intraocular pressure and/or hypoxia result in oxidative stress, and that leads to mitochondrial dysfunction and subsequent energy failure during glaucomatous ONH degeneration (Li et al., 2015). In fact, human astrocytes *in vitro* from ONH tissues in patients with POAG showed evidence of oxidative stress, bioenergetic dysfunction, or mitochondrial dysfunction by compromising mitochondrial network (Ju et al., 2015). In line with these findings, our recent study has demonstrated that oxidative stress induced a significant loss of mitochondrial mass and impairment of mitochondrial network and OXPHOS system in ONH astrocytes by decreasing mitochondrial number and volume. In contrast, inhibition of oxidative stress by coenzyme Q10, an attractive antioxidant and neurotherapeutic agent, increased mitochondrial mass by triggering mitochondrial biogenesis and improved bioenergetic function by preserving OXPHOS and ATP production in ONH astrocytes (Noh et al., 2013). These findings suggest that oxidative stress-mediated mitochondrial dysfunction or alteration is likely to be an important pathophysiological mechanism in the dysfunction of ONH astrocyte in glaucoma progression.

Since glutamate excitotoxicity is a well-known source of oxidative stress, our recent study showed that that glaucomatous ONH astrocytes from patients with POAG upregulated expression levels of N-methyl-D-aspartate receptor proteins, as well as induced an extensive mitochondrial fragmentation (Ju et al., 2015). Using a chronic mouse model of glaucoma, aged DBA/2J mice, we have further demonstrated that mitochondrial loss by the impaired mitochondrial network is associated with dysfunctional ONH astrocytes in glaucomatous neurodegeneration (Ju et al., 2015). In this study, we have importantly proposed that increased mitochondrial fission and volume by blocking glutamate excitotoxicity protect glaucomatous ONH astrocytes and that modulation of mitochondrial network is considered to be critical to developing therapeutic strategies for protecting glaucomatous ONH astrocytes that lead to the preservation of RGC axons in glaucomatous neurodegeneration.

cAMP/PKA pathway and mitochondrial protection in optic nerve head astrocytes: In healthy cells, mitochondria are autonomous and morphologically dynamic organelles that structurally reflect a precise balance of ongoing dynamics, fission and fusion, within a cell. This balance is regulated by a family of dynamin-related GTPases that exert opposing effects. The mitofusins (Mfns) and optic atrophy type 1 (OPA1) are required for mitochondria fusion, whereas dynamin-related protein 1 (Drp1) regulates mitochondrial fission.

Transient induction of mild oxidative stress (relatively lower concentration of H_2O_2) compromises mitochondrial bioenergetics by decreasing both basal and maximal respiration, and this leads to the deficit of Drp1 protein expression, accompanied by compromised mitochondrial activity and cell viability (Ju et al., 2019). Furthermore, these alterations are relevant to the changes of expression levels of mitochondrial fusion proteins (OPA1 as well as Mfn1 and 2) in ONH astrocytes (Ju et al., 2019). Drp1 deficiency contributes to bioenergetic dysfunction in axonal mitochondria, leading to significant defects in maintaining normal ATP level and synaptic vesicle cycling. Thus, our findings reflect that oxidative stress-induced Drp1 defect in ONH astrocytes may play a critical role in the impairment of mitochondrial bioenergetics, contributing to dysfunction of ONH astrocytes.

cAMP links to the regulation of mitochondrial dynamics and OXPHOS in mammalian cells. However, the relevance between cAMP/PKA signaling and mitochondrial dynamics in ONH astrocytes is poorly understood. In contrast to oxidative stress-induced Drp1 defect in ONH astrocytes, an elevated intracellular cAMP level significantly increased Drp1 protein expression in ONH astrocytes, but not mitochondrial fusion proteins such as OPA1as well as Mfn1 and 2 (Ju et al., 2019). These results raised a possibility that Drp1 could be a key player in the alteration of mitochondrial dynamics of ONH astrocyte dysfunction in response to oxidative stress and/or intracellular cAMP elevation. It has been reported that cAMP/PKA phosphorylation of Drp1 regulates its GTPase activity and mitochondrial morphology and that phosphorylation of Drp1 at serine 637 inhibits mitochondrial division (Chang and Blackstone, 2017). Based on our finding of extensive mitochondrial fragmentation in ONH astrocytes by combined oxidative stress and cAMP elevation (Ju et al., 2019), it is possible that combined oxidative stress and cAMP elevation may induce mitochondrial fragmentation in ONH astrocytes via dephosphorylated Drp1 at serine 637. However, we believe that further studies will be required to determine the relationship between cAMP/PKA pathway and Drp1 phosphorylation in ONH astrocytes against glaucomatous insults such as oxidative stress.

Also, our findings showed that elevated cAMP induced a significant reduction of total OPA1 as well as Mfn 1 and 2 protein expression in ONH astrocytes exposed to oxidative stress, while no statistically significant difference was detected

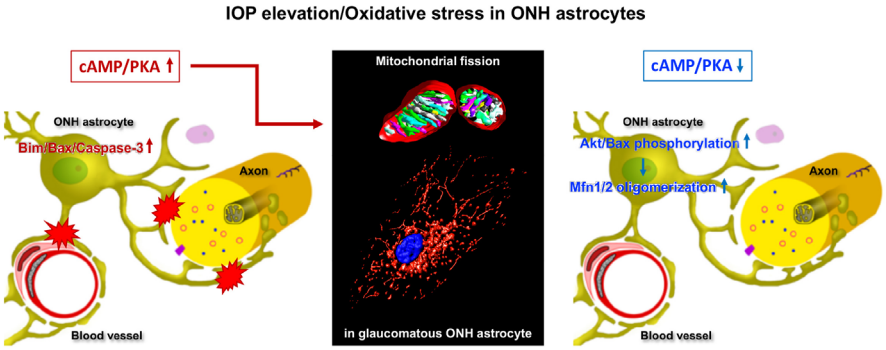

Figure 1 | A hypothetical model for the role of the cAMP/PKA pathway on mitochondrial dysfunction and protection in ONH astrocytes against glaucomatous insults.
Elevated intraocular pressure contributes to cAMP elevation, mitochondrial dysfunction, and caspase-3 activation in ONH astrocytes. Elevated intracellular cAMP exacerbates abnormality of mitochondrial dynamics (extensive mitochondrial fission and loss) in ONH astrocytes exposed to oxidative stress. PKA inhibition protects ONH astrocytes against oxidative stress combined with cAMP elevation via Akt/Bax phosphorylation and Mfn1/2 oligomerization. cAMP: Cyclic adenosine 3′,5′-monophosphate; IOP: intraocular pressure; ONH: optic nerve head; PKA: protein kinase A.

in the total level of Drp1 protein expression compared with control (Ju et al., 2019). Because this surprising alteration of mitochondrial dynamics was correlated with a worse reduction of mitochondrial activity and cell viability in the ONH astrocytes, our results suggest that elevated level of intracellular cAMP may exacerbate vulnerability to mitochondrial dysfunction in ONH astrocytes exposed to oxidative stress via the impaired activity of mitochondrial fusion during glaucoma progression.

Since glaucomatous damage in human, rat, and mouse ONH astrocytes is associated with extensive mitochondrial fragmentation and loss (Noh et al., 2013; Ju et al., 2015), no evidence for the molecular mechanism of impaired mitochondrial fusion activity was reported in glaucomatous ONH astrocytes. Mfn1 and 2 are GTPase dynamin-like proteins of the outer mitochondrial membrane, which are essential for fusion activity in the mitochondria of human cells. Overexpression of Mfn2 protects neuronal cells in the brain against ischemia/reperfusion, and activated Mfn2 protects the mitochondria by inhibiting Bax activation, cytochrome c release, and permeability transition. Our emerging evidence showed that PKA inhibition preserved mitochondrial network and enhanced ONH astrocyte survival by increasing the oligomerization of both Mfn1 and 2 against oxidative stress combined with cAMP elevation (Ju et al., 2019), suggesting that increased activity of Mfn1 and 2 oligomerization may have therapeutic potential to protect ONH astrocyte against glaucomatous insults such as oxidative stress.

Our recent study proposed that transient induction of mild oxidative stress may trigger an endogenous defense mechanism by decreasing the intracellular level of cAMP in ONH astrocytes (Shim et al., 2018). Interestingly, mild oxidative stress showed a lower level of Bax protein expression, indicating that an endogenous compensatory mechanism induced by mild oxidative stress is associated with a temporary reduction of active Bax protein expression in ONH astrocytes. While Bax does not alter the activity of Mfn1-Mfn2 *trans* heterotypic complexes, soluble Bax positively regulates mitochondrial fusion activity by Mfn2 homotypic complexes on mitochondria. Since cytoplasmic Bax is endogenously phosphorylated, most likely at serine184, regulating heterodimerization of Bax with anti-apoptotic Bcl-2 family members (Gardai et al., 2004), the soluble, nonoligomerized form of Bax is the primary cytosolic regulator of mitochondrial fusion (Hoppins et al., 2011).

We have shown that PKA inhibition could increase not only Akt phosphorylation at serine 473 but also Bax phosphorylation at serine184 in ONH astrocytes against oxidative stress combined with cAMP elevation. Akt inhibits a conformational change in the Bax protein and its translocation to mitochondria, leading to prevent mitochondrial dysfunction and cell death. Because Akt regulates Bax phosphorylation at serine184 and inhibits Bax effects on the mitochondria, we have proposed a link of the pathological pathway between cAMP/PKA activation and Akt/Bim/Bax-mediated intrinsic cell death in ONH astrocyte dysfunction. Thus, our results strongly suggest the notion that Akt/Bax phosphorylation by inhibiting the cAMP/PKA pathway would be an important defense mechanism in glaucomatous ONH astrocyte dysfunction.

Our study provides evidence that the activation of the cAMP/PKA pathway has a critical role in the impairment of mitochondrial dynamics and bioenergetics of ONH astrocytes. Moreover, elevated cAMP exacerbates mitochondrial dysfunction to oxidative stress in ONH astrocytes. Inhibition of intracellular cAMP/PKA pathway can protect ONH astrocytes by increasing Akt/Bax phosphorylation and Mfn1/2 oligomerization. Since overexpression of OPA1 or inhibition of Drp1 protects RGC and its axons by preserving mitochondrial network and function in glaucomatous neurodegeneration, our findings importantly suggest that modulation of cAMP/PKA pathway or mitochondrial network may have therapeutic potential to protect ONH astrocytes by preserving mitochondrial function in glaucomatous neurodegeneration (**Figure 1**). Therefore, it would be useful to further determine the therapeutic potential of Mfn1/2-mediated protection in glaucomatous ONH astrocytes in future studies.

Keun-Young Kim, Won-Kyu Ju*
National Center for Microscopy and Imaging Research and Department of Neurosciences, University of California San Diego, La Jolla, CA, USA (Kim KY)
Hamilton Glaucoma Center and Shiley Eye Institute, The Viterbi Family Department of Ophthalmology, University of California San Diego, La Jolla, CA, USA (Ju WK)
***Correspondence to:** Won-Kyu Ju, PhD, wju@ucsd.edu.
https://orcid.org/0000-0003-1117-4834 (Won-Kyu Ju)
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