

● PERSPECTIVE

NLRP3 inflammasome in retinal ganglion cell loss in optic neuropathy

In neurodegenerative diseases, neuroinflammatory responses are often activated in resident immune cells in the central nervous system (CNS) (Schroder and Tschopp, 2010). Optic neuropathy refers to dysfunction and degeneration of retinal ganglion cells (RGCs) and their axons, which is often induced by optic nerve injury or glaucomatous insult. Studies, including ours, suggested that the optic nerve insult activated the NLRP3 inflammasome (NOD-like receptors, abbreviated as NLRs, Pyrin domain containing 3, also known as NALP3) in retinal microglial cells (Chi et al., 2014; Puyang et al., 2016). We have applied non-invasive high-resolution imaging to track RGC survival *in vivo* and demonstrated that knocking out NLRP3 gene delayed RGC loss following the optic nerve crush injury (Puyang et al., 2016). In this article, we discussed the role of NLRP3 inflammasome in RGC death in eye diseases.

Activation of NLRP3 inflammasome following optic nerve crush injury or glaucomatous insult: The insults to the optic nerve are categorized into two major types: (1) microbial pathogen-related, and (2) injury- or stress-induced insults (Soto and Howell, 2014). The pattern recognition receptors (PRRs) detect (1) pathogen-associated molecular patterns (PAMPs), such as microbial secretion systems, components of cell wall, and nucleic acid in pathogens, and (2) danger-associated molecular patterns (DAMPs), which include uric acid, ATP, and heat shock proteins (HSPs), *etc* (Schroder and Tschopp, 2010; Soto and Howell, 2014). There are two major categories of PRRs in microglia and other immune cells: Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), which are located in the plasma membrane and endosomes (Schroder and Tschopp, 2010). All TLRs (TLR1 to TLR13) are found in microglial cells, but only less than half of TLRs (TLR2-5 and TLR9) are expressed in astrocytes (Soto and Howell, 2014). The second type of PRRs includes RIG-I-like receptors (RLRs), AIM2-like receptors (ALRs), and NLRs, which are found in intracellular compartments (Schroder and Tschopp, 2010). Both TLRs and NLRs could recognize exogenous pathogen-related and endogenous danger signal molecules.

The NLR family has more than 22 members in mice, among which NLRP3 is best known to form the inflammatory multi-protein complex and platform *i.e.*, NLRP3 inflammasome (Schroder and Tschopp, 2010). Importantly, NLRP3 inflammasome acts as a sensor for the primary inflammatory signal and amplifies the signal in the CNS. NLRP3 inflammasome was mainly activated in microglial cells and macrophages in the brain and the retina (Schroder and Tschopp, 2010; Soto and Howell, 2014; Puyang et al., 2016). Previous studies suggested that the initial damage of the neural retina triggered glial cells to activate pro-inflammatory responses against danger. For example, DAMPs activated NLRP3 inflammasome and caspase-8 pathways induced by the temporary retinal ischemia (Chi et al., 2014). Diabetes and neurotoxicity also induced ER stress, which in

turn activated NLRP3 inflammasome in rodent Müller glia; and extracellular ATP could also activate the P2X7 receptors and NLRP3 inflammasome in macrophages (see discussion in Puyang et al., 2016).

Recent evidences supported that microglial cells are activated in response to glaucomatous insult or optic nerve injury (Bosco et al., 2011, 2012; Chi et al., 2014; Puyang et al., 2016). Microglial cells labeled by CX3CR1-GFP transgene (green fluorescent protein driven by the promoter of C-X3-C motif chemokine receptor 1 which is highly expressed in microglial cells) was examined in both optic nerve crush model and acute ocular hypertension model (Liu et al., 2012). Liu et al. (2012) showed that microgliosis went up in the first week following the optic nerve insult or acute intraocular pressure (IOP) elevation and then dropped to baseline by the end of the fourth week. Another study on DBA/2 mice, a mouse model for spontaneous secondary glaucoma, showed that the microglia activation peaked by 3 months comparing to age-matched healthy controls (Bosco et al., 2011). Our recent study demonstrated that NLRP3 was up-regulated in microglial cells upon partial optic nerve crush injury and the activation of NLRP3 propagated from the injury site to the entire retina within 1 day (Puyang et al., 2016).

NLRP3 inflammasome consists of NLRP3, the scaffold protein ASC (apoptosis associated speck-like protein), and proinflammatory pro-caspase-1. Once the NLRP3 inflammasome is activated, pro-caspase-1 is auto-cleaved to become mature caspase-1. Caspase-1 then cleaves proinflammatory cytokines such as pro-interleukine (IL)-1 β to mature IL-1 β (Schroder and Tschopp, 2010; Soto and Howell, 2014). We showed that both caspase-1 and IL-1 β were up-regulated following the optic nerve crush injury in wildtype retina, and their activation was reduced in the NLRP3 knockout mice (Figure 2 in Puyang et al., 2016). Our study is consistent with an early finding that pharmacological inhibition of caspase-1 reduced the expression of IL-1 β to baseline level following acute IOP elevation (Chi et al., 2014). Chi et al. (2014) colleagues also showed that inhibition to caspase-8 further decreased IL-1 β level to below baseline; and knocking out TLR4 reduced the RGC damage and inhibited the hyperactivity of caspase-8 signaling, suggesting that IL-1 β can be activated *via* both NLRP3-caspase-1-dependent and -independent pathways. It is likely that TLRs and NLRPs interact with each other to up-regulate the maturation of inflammatory cytokines (Schroder and Tschopp, 2010; Soto and Howell, 2014). Together these studies support that NLRP3 inflammasome can be activated upon the optic nerve crush injury or glaucomatous insult which may lead to subsequent neurodegeneration.

***In vivo* imaging to monitor RGC survival following optic nerve injury:** Recent studies on morphological and functional degeneration of RGCs suggested that different types of RGCs exhibit different susceptibility to the glaucomatous insult (Puyang et al., 2015). For example, RGC dendritic structure examined by confocal imaging in fixed tissues exhibited type-dependent degeneration in different models of experimental glaucoma (reviewed by Puyang et al., 2015). Leung and his colleagues applied a blue-light confocal scanning laser ophthalmoscope (bCSLO) to track RGC survival *in vivo* using the Thy-1-YFP-16 mice, in which yellow fluorescent protein driven by the Thy-1 promoter was expressed

in RGC and/or amacrine cells (Puyang et al., 2016; Leung et al., 2011). Their results suggested that RGC survival rate varied in different types, ranging from 50% to 100% at 2 weeks and 20% to 80% at 4 weeks post optic nerve crush (Leung et al., 2011). Using a fluorescent fundus Micron III system, we imaged RGCs and their axons of Thy-1-YFP-H mice following the optic nerve crush injury (Puyang et al., 2016). We showed that the overall RGC survival rate decreased from 53% at 1 week, to 30% at 2 weeks and 13% at 4 weeks post injury (Puyang et al., 2016). These *in vivo* imaging systems eliminated the sampling variations from mouse to mouse, a common concern in RGC quantification with fixed tissue. Furthermore, we showed that RGC and axon loss was delayed in NLRP3 knockout mice for about 1 week, supporting a negative role of activation of NLRP3 inflammasome in RGC survival (Puyang et al., 2016).

In vivo imaging also offers the opportunity to address the subtype- or location-dependent RGC survival and the dendritic remodeling of individual cells post injury. Leung's studies demonstrated that RGCs with larger dendritic field size and more distal site tended to exhibit less susceptibility to the insult (Leung et al., 2011). We examined the number of RGC axons following optic nerve crush injury and found no significant difference between central and peripheral areas of the retina, though we cannot rule out the possibility that certain RGC types resided in a particular area are less resistance to the insult than others (Puyang et al., 2016). One encouraging future direction would be to use transgenic mouse lines with specific RGC subtypes labeled to study RGC subtype loss at a fine granularity following optic nerve injury or glaucomatous insult.

NLRP3 inflammasome in eye diseases: Not until recent decade the inflammasomes were found associated with different eye diseases, yet studies suggested a controversial role of NLRP3 inflammasome in different mouse disease models (Celkova et al., 2015). Activation of the NLRP3 inflammasome led to maturation of interleukins, which have been documented in patients with eye diseases (Celkova et al., 2015). In rodent model for dry-age-related macular degeneration (AMD), in which geographic atrophy (GA) was developed with no obvious bleeding, blocking NLRP3 or downstream IL-18 and IL-1 β reduced the degeneration, suggesting a destructive role of NLRP3 inflammasome (Celkova et al., 2015). On the other hand, NLRP3 inflammasome was shown to have a protective role in wet/neovascular AMD, where abnormally growing blood vessels (choroidal neovascularization) cause hemorrhage between the retinal pigment epithelium and the foveal photoreceptors. Activation of NLRP3 inflammasome up-regulated IL-18, which in turn down-regulated the synthesis of vascular endothelial growth factor (VEGF) to provide protection in a mouse model of wet-AMD; and ablation of NLRP3 induced more severe neovascularization and subretinal hemorrhaging (Celkova et al., 2015).

In DBA/2 mice, an early microglial activation was found to peak before the progression of the disease (Bosco et al., 2011, 2012). Another study characterized the reactions of retinal astrocytes in response to the optic nerve insult, suggesting that dynamic changes of astrocytes organization also contributed to the onset and progression of RGC degeneration (Formichella et al., 2014). These studies suggested that neuroinflammatory responses were initiated at the early stages of experimental glaucoma, which may lead to subsequent

RGC degeneration and death. Future work is needed to characterize the NLRP3 inflammasome-mediated neuroinflammatory pathways in RGC death in glaucoma.

Conclusions: Combined with mouse transgenic lines with RGCs labeled by fluorescent proteins, the *in vivo* imaging provides an excellent model system to monitor RGC survival. Using this model system, we have demonstrated that the RGC survival was extended in NLRP3 knockout mice post optic nerve crush injury. Our study added solid evidence that NLRP3 inflammasome acts as a sensor to detect the optic nerve injury and then amplify the damage signal in the entire retina, leading to subsequent RGC loss. It provides opportunities for developing drugs targeting the NLRP3 inflammasome to better preserve vision of patients with optic neuropathy.

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References

- Bosco A, Steele MR, Vetter ML (2011) Early microglia activation in a mouse model of chronic glaucoma. *J Comp Neurol* 519:599-620.
- Bosco A, Crish SD, Steele MR, Romero CO, Inman DM, Horner PJ, Calkins DJ, Vetter ML (2012) Early reduction of microglia activation by irradiation in a model of chronic glaucoma. *PLoS One* 7:e43602.
- Celkova L, Doyle SL, Campbell M (2015) NLRP3 inflammasome and pathobiology in AMD. *J Clin Med* 4:172-192.
- Chi W, Li F, Chen H, Wang Y, Zhu Y, Yang X, Zhu J, Wu F, Ouyang H, Ge J, Weinreb RN, Zhang K, Zhuo Y (2014) Caspase-8 promotes NLRP1/NLRP3 inflammasome activation and IL-1 β production in acute glaucoma. *Proc Natl Acad Sci U S A* 111:11181-11186.
- Formichella CR, Abella SK, Sims SM, Cathcart HM, Sappington RM (2014) Astrocyte reactivity: a biomarker for retinal ganglion cell health in retinal neurodegeneration. *J Clin Cell Immunol* 5:188.doi:10.4172/2155-9899.1000188.
- Krizaj D, Ryskamp DA, Tian N, Tezel G, Mitchell CH, Slepak VZ, Shestopalov VI (2014) From mechanosensitivity to inflammatory responses: new players in the pathology of glaucoma. *Curr Eye Res* 39:105-119.
- Leung CK, Weinreb RN, Li ZW, Liu S, Lindsey JD, Choi N, Liu L, Cheung CY, Ye C, Qiu K, Chen LJ, Yung WH, Crowston JG, Pu M, So KF, Pang CP, Lam DS (2011) Long-term *in vivo* imaging and measurement of dendritic shrinkage of retinal ganglion cells. *Invest Ophthalmol Vis Sci* 52:1539-1547.
- Liu S, Li ZW, Weinreb RN, Xu G, Lindsey JD, Ye C, Yung WH, Pang CP, Lam DS, Leung CK (2012) Tracking retinal microgliosis in models of retinal ganglion cell damage. *Invest Ophthalmol Vis Sci* 53:6254-6262.
- Puyang Z, Chen H, Liu X (2015) Subtype-dependent morphological and functional degeneration of retinal ganglion cells in mouse models of experimental glaucoma. *J Nat Sci* 1:e103.
- Puyang Z, Feng L, Chen H, Liang P, Troy JB, Liu X (2016) Retinal ganglion cell loss is delayed following optic nerve crush in *nlrp3* knockout mice. *Sci Rep* 6:20998.
- Schroder K, Tschopp J (2010) The inflammasomes. *Cell* 140:821-832.
- Soto I, Howell GR (2014) The complex role of neuroinflammation in glaucoma. *Cold Spring Harb Perspect Med* 4:a017269.