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Review

Dock3-NMDA receptor interaction as a target for glaucoma therapy

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Summary. Glaucoma is a neurodegenerative disease of the eye and it is one of the major causes of blindness. Glaucoma is usually associated with elevated intraocular pressure (IOP) and the current therapy focuses on reduction of IOP. However, neuroprotective strategies could also be beneficial for treatment of glaucoma because the pathology of the disease involves retinal ganglion cell (RGC) death and damage to the optic nerve. Dedicator of cytokinesis 3 (Dock3) is an atypical guanine exchange factor (GEF) that belongs to a family of Dock proteins, Dock1-11. Dock3 exerts neuroprotective effects on the retina and optic nerve, and studies revealed that some of the Dock3-mediated effects are GEF-independent. One of these mechanisms is that Dock3 directly binds to the GluN2B subunit of the *N*-methyl-D-aspartate (NMDA) receptor. Upon stimulation by NMDA or optic nerve crush, overexpression of Dock3 promotes internalization and degradation of the NMDA receptor in the retina *in vivo*. It is suggested that this process is mediated by inhibition of Fyn, a Src family tyrosine kinase. Reduction in NMDA receptor expression results in decreased excitotoxic damage and oxidative stress, thereby promoting RGC survival. In this review, we discuss the therapeutic potential of neuroprotection for glaucoma and the effects of Dock3 on NMDA receptors. We also discuss apoptosis signal-regulating kinase 1 (ASK1), a member of mitogen-activated protein kinase kinase kinase that is a key regulator of cellular responses to oxidative stress, as an innovative therapeutic target for glaucoma.

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Introduction

Glaucoma is a neurodegenerative disease of the eye and it is one of the leading causes of vision loss. It is estimated that by 2020, more than 80 million people will be affected worldwide, with at least 6 to 8 million of them becoming bilaterally blind (Quigley and Broman, 2006). Glaucoma is characterized by progressive degeneration of retinal ganglion cells (RGCs) and their axons, namely the optic nerve, usually associated with elevated intraocular pressure (IOP). Currently, pharmacological intervention for glaucoma therapy is limited to reduction of IOP, which is the only treatment with successful therapeutic outcome. In preclinical research, animal models of glaucoma have contributed greatly in understanding glaucoma pathology as well as for examining potential therapeutic candidates. While several inherited and experimentally-induced animal models of high IOP glaucoma have been available for some time, for example the inherited model DBA/2J mice (John et al., 1998), mouse models of normal tension glaucoma (NTG), a subset of glaucoma that is not associated with increased IOP, have only recently become available. The general concept is that the number of NTG patients is small relative to the total number of glaucoma patients; however, there is an unexpectedly high prevalence of NTG in Japan and other Asian countries (Iwase et al., 2004; Kim et al., 2011). Harada et al. discovered that deletion of glutamate transporters, glutamate/aspartate transporter (GLAST) or excitatory amino acid carrier 1 (EAAC1), exhibits spontaneous RGC death and optic nerve degeneration

without elevation of IOP, a pathology that is similar to NTG (Harada et al., 2007). GLAST is expressed in Müller glia in the retina and clears excess glutamate from the synapse, thus preventing excitotoxic damage on surrounding retinal neurons (Harada et al., 1998). In addition, glutamate that is transported into Müller glia by the glutamate uptake process, together with cysteine and glycine, is converted to glutathione (GSH), a major antioxidant in the retina (Reichelt et al., 1997). Increased oxidative stress is one of the risk factors in glaucoma and consistently, the plasma GSH level is decreased in glaucoma patients (Gherghel et al., 2005, 2013). In GLAST KO mice, decreased glutamate uptake into Müller glia leads to reduced GSH production (Harada et al., 2007), indicating that GLAST KO mice exhibit key pathological features of NTG, including lack of IOP elevation, RGC loss and optic nerve atrophy as a result of glutamate neurotoxicity and increased oxidative stress.

Glaucoma and neuroprotection

While the current glaucoma therapy focuses on reduction of IOP, the therapeutic potential of neuroprotection of RGCs is raising awareness (Levin and Peeples, 2008; Osborne and del Olmo-Aguado, 2013; Tian et al., 2015; Gossman et al., 2016). In fact, findings from preclinical studies points favourably to this notion (Cheung et al., 2008; Johnson et al., 2014; Namekata et al., 2014a; Kimura et al., 2015; Noro et al., 2015). Indeed, one of the neuroprotective agents, memantine, has produced some outstanding results in preclinical studies (Hare et al., 2004a,b; Harada et al., 2007; Ju et al., 2009; Atorf et al., 2013). Memantine is a synthetic compound that acts as an antagonist at the N-methyl-D-aspartate (NMDA) receptor, an ionotropic glutamate receptor with high calcium permeability (Bormann, 1989; Chen et al., 1992). In the retina, NMDA receptors are expressed in neurons in the inner nuclear layer (INL) and the ganglion cell layer (GCL) (Brandstatter et al., 1994, 1998). Overstimulation of NMDA receptors in neurons causes excessive elevation in the intracellular calcium level, which causes excitotoxic cell damage and death. Therefore, NMDA antagonists are promising candidates for diseases that involve neuronal cell damage or death associated with excitotoxicity, for example, various neurodegenerative diseases including amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease and glaucoma (Seki and Lipton, 2008; Dong et al., 2009; King et al., 2016). In 2003, the US food and drug administration (FDA) approved memantine (its brand name Namenda) to be used for treatment of moderate to severe symptoms of Alzheimer's disease. In contrast, memantine failed the clinical trials for use in glaucoma in 2008. This was a disappointing result, but this should not discourage the idea that neuroprotection is a potential therapeutic strategy for glaucoma (Osborne, 2009). Active research into neuroprotection as a potential therapeutic strategy for glaucoma continues.

Dedicator of cytokinesis 3 (Dock3)

Dedicator of cytokinesis (Dock) proteins are atypical guanine exchange factors (GEFs) that regulate the activation of Rho GTPases. They play important roles in actin polymerization, migration and cell adhesion (Cote and Vuori, 2007; Laurin and Cote, 2014; Namekata et al., 2014a). To date, 11 family members have been identified (Dock1-11; Table 1). Dock3 was initially identified as a presenilin binding protein, and its expression is decreased in the soluble cellular component in Alzheimer's disease brain (Kashiwa et al., 2000). Subsequent studies suggested that Dock3 may play a regulatory role in tau phosphorylation and in the formation of neurofibrillary tangles in Alzheimer's disease brain (Chen et al., 2001), but the exact roles of Dock3 in Alzheimer's disease remain unknown. Dock3 is specifically expressed in the central nervous system (CNS) (Namekata et al., 2004) and loss of Dock3 induces cytoskeleton disorganization and axonal dysfunction in mice (Chen et al., 2009). Genetic family studies have identified a Dock3 gene disruption in attention deficit hyperactivity disorder (de Silva et al., 2003). In the retina, Dock3 is expressed in RGCs and it stimulates axon regeneration following optic nerve

Table 1. Targeted small G proteins, tissue distribution and cellular function of the Dock family.

Dock	GEF activity	Expression	Cellular function	Reference
Dock1	Rac	ubiquitous	axon guidance; myoblast fusion	Katoh and Negishi, 2003; Li et al., 2008; Laurin et al., 2008
Dock2	Rac	lymphocytes	lymphocyte migration	Fukui et al., 2001
Dock3	Rac	CNS	axon regeneration; neuroprotection	Namekata et al., 2010, 2012a,b, 2013
Dock4	Rac	CNS, lung	dentrite development; lung adenocarcionoma invasion	Ueda et al., 2013; Yu et al., 2015
Dock5	Rac	ubiquitous	myoblast fusion	Laurin et al., 2008
Dock6	Rac/Cdc42	ubiquitous	axon outgrowth	Miyamoto et al., 2007
Dock7	Rac/Cdc42	ubiquitous	neurogenesis; schwann cell migration	Yang et al., 2012; Yamauchi et al., 2008
Dock8	Cdc42	lymphocytes	T-cell and B-cell development	Randall et al., 2009
Dock9	Cdc42	ubiquitous	dentrite development	Kuramoto et al., 2009
Dock10	Rac/Cdc42	ubiquitous	melanoma cell invasion	Gadea et al., 2008; Ruiz-Lafuente et al., 2015
Dock11	Cdc42	lymphocytes	lymphocite development and migration	Sakabe et al., 2012; Matsuda et al., 2015

transection (Namekata et al., 2010, 2012a, 2014a). Dock3 activates Rac1 and it acts downstream of brain derived neurotrophic factor (BDNF) and its receptor TrkB. When the BDNF-TrkB pathway is activated, Dock3 is translocated to the cell surface where it activates various signalling cascades and promotes neuroregeneration (Namekata et al., 2004, 2010, 2012a,b). Furthermore, Dock3 is also expressed in oligodendrocytes and overexpression of Dock3 protects myelin in the cuprizone-induced demyelination model (Namekata et al., 2014b). Taken together, increased Dock3 expression may have a therapeutic value in neurodegenerative diseases.

Dock3 regulates retinal NMDA receptor activity

Interestingly, Dock3 possesses a binding site for the GluN2B subunit (Fig. 1) and it interacts directly with the intracellular C-terminus of the GluN2B subunit (Namekata et al., 2013), suggesting that Dock3 and NMDA receptors may have a regulatory interaction. The NMDA receptor is one of the ionotropic glutamate receptors and it is a heterotetramer consisting of subunits GluN1 and GluN2A-D. This ligand-gated cation channel is usually composed of two GluN1 subunits, which bind its co-agonist glycine, and at least two GluN2 (A–D) subunits, which bind glutamate. NMDA receptor subunits show different pharmacological properties and the subunit composition strongly influences the functional properties of the NMDA receptor (Cull-Candy et al., 2001; Paoletti et al., 2013). The intracellular C-terminal domain of GluN2B is known to regulate internalization and degradation of the GluN2B-containing NMDA receptors (Roche et al., 2001); therefore one speculates that Dock3 may have a regulatory role on the NMDA receptor function by binding to the C-terminus of the GluN2B subunit. The GluN2B C-terminal tail is phosphorylated by the tyrosine kinase Fyn at Tyr1252, Tyr1336 and Tyr1472, of which, phosphorylation of Tyr1472 plays important roles including stabilization of the NMDA receptor complex at the plasma membrane (Nakazawa et al., 2001; Salter and Kalia, 2004). Interestingly, Dock3 directly interacts with Fyn and overexpression of Dock3 reduces the GluN2B protein expression following intravitreal injection of NMDA (Namekata et al., 2010; Namekata et al., 2013). Dock3 preferentially binds to the active form of Fyn (Namekata et al., 2010), suggesting that this interaction could affect the enzymatic transition state of Fyn. In addition, Dock3 directly binds to GluN2B and stimulates NMDA-induced GluN2B degradation in the mouse retina, resulting in the protection of RGCs from excitotoxic damage (Namekata et al., 2013). Moreover, a reduction in retinal GluN2B expression is observed in mice with overexpression of Dock3 in an optic nerve injury model, which mimics many features of glaucoma (Semba et al., 2014). These findings suggest that upon stimulation, Dock3 may modulate Fyn activity and promote NMDA receptor internalization, which could reduce excitotoxic damage to RGCs (Fig. 2). Indeed, when Dock3 is overexpressed in GLAST KO mice, Tyr1472 phosphorylation of the GluN2B was reduced and RGC loss was ameliorated (Namekata et al., 2013), supporting the notion that Dock3 suppresses Tyr1472 phosphorylation, thereby promoting NMDA receptor internalization.

Recent studies have shown that the protein tau, which is implicated in the pathogenesis of Alzheimer's disease, promotes Fyn-mediated GluN2B stabilization at the plasma membrane, resulting in enhanced NMDA receptor-mediated neurotoxicity (Amadoro et al., 2006; Ittner et al., 2010). Dock3 undergoes characteristic changes in the Alzheimer's disease brain (Kashiwa et al., 2000), and so it is possible that Dock3 inhibits Fyn-mediated GluN2B stabilization at the plasma membrane and exerts neuroprotective effects on the Alzheimer's disease brain. Taken together, the Dock3-GluN2B interaction may play an important role in regulating NMDA receptor function during disease and thus, manipulation of this interaction may be a novel target for various neurodegenerative diseases including glaucoma and Alzheimer's disease. Further studies are required to reveal the precise mechanisms for Dock3-mediated degradation of the NMDA receptors.

Dock3 and oxidative stress

In addition to glutamate neurotoxicity, oxidative stress is recognized as a common pathological factor in

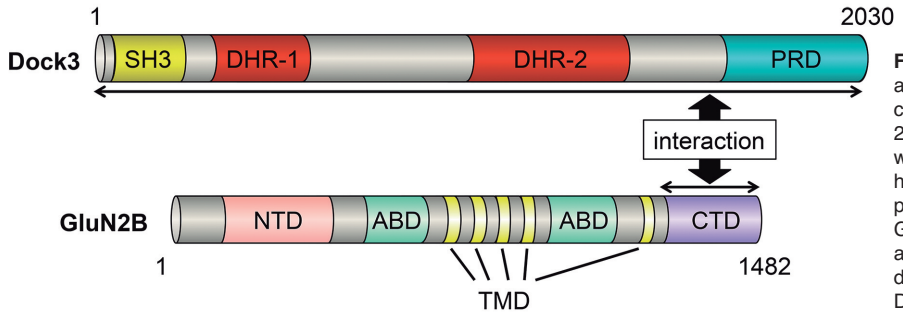

Fig. 1. Domain structures of Dock3 and GluN2B and their interaction sites. Dock3 possesses the conserved DHR-1 domain that precedes the DHR-2 domain (catalytic domain for small G proteins), which is characteristic of the Dock family. It also has an SH3 domain at the N-terminus and a proline rich domain (PRD) at the C-terminus. GluN2B possesses the N-terminal domain (NTD), agonist binding domain (ABD), transmembrane domain (TMD) and C-terminal domain (CTD). Dock3 binds to the CTD of GluN2B.

many neurodegenerative diseases, including glaucoma (Osborne and del Olmo-Aguado, 2013). As mentioned earlier, the level of the antioxidant GSH is reduced in glaucoma patients and in GLAST KO mice, indicating that the oxidative stress level is increased in glaucoma (Gherghel et al., 2005, 2013; Harada et al., 2007). We previously reported that apoptosis signal-regulating kinase 1 (ASK1), a member of mitogen-activated protein kinase kinase kinase (MAP3K), could be a therapeutic target for glaucoma (Harada et al., 2006, 2010). ASK1 plays key roles in cellular responses to oxidative stress, endoplasmic reticulum stress and proinflammatory cytokines (Nishitoh et al., 2008; Hattori et al., 2009; Guo et al., 2010). Deletion of the ASK1 gene (ASK1 KO) from GLAST KO mice (GLAST/ASK1 double KO mice) revealed that ASK1 is associated with progressive RGC loss, glaucomatous optic nerve degeneration and visual impairment in GLAST KO mice (Harada et al., 2010). Moreover, ASK1 KO mice show reduced proinflammatory responses in a mouse model of multiple sclerosis and reduced RGC death following optic nerve injury (Guo et al., 2010; Katome et al., 2013). Therefore, inhibition of the ASK1 activity holds therapeutic potential for various neurodegenerative diseases including glaucoma and multiple sclerosis (Kawarazaki et al., 2014). Interestingly, overexpression of Dock3 protects RGCs from oxidative stress *in vitro* and decreases activation of the ASK1-p38 pathway following optic nerve injury *in vivo* (Namekata et al., 2013; Semba et al., 2014). Although the detailed mechanisms are unclear, it is possible that Dock3 may prevent oxidative stress-induced RGC death by suppression of the ASK1 pathway. In addition, stimulation of NMDA receptors leads to superoxide production and neurotoxicity in neurons (Lafon-Cazal et al., 1993; Brennan et al., 2009). Therefore, it is possible that Dock3 could reduce oxidative stress indirectly by attenuating NMDA receptor activation.

Concluding remarks and future perspectives

Neuroprotection is a promising therapeutic strategy for glaucoma. However, combined therapy, for example with IOP reduction or protection of the optic nerve, may be required to produce clear beneficial outcome in patients. Dock3 is exclusively expressed in the CNS and therefore from a pharmaceutical point of view, it is a very attractive drug target. Currently, we are interested in overexpression of Dock3 using adeno-associated virus and exploring its use for glaucoma therapy. Modulation

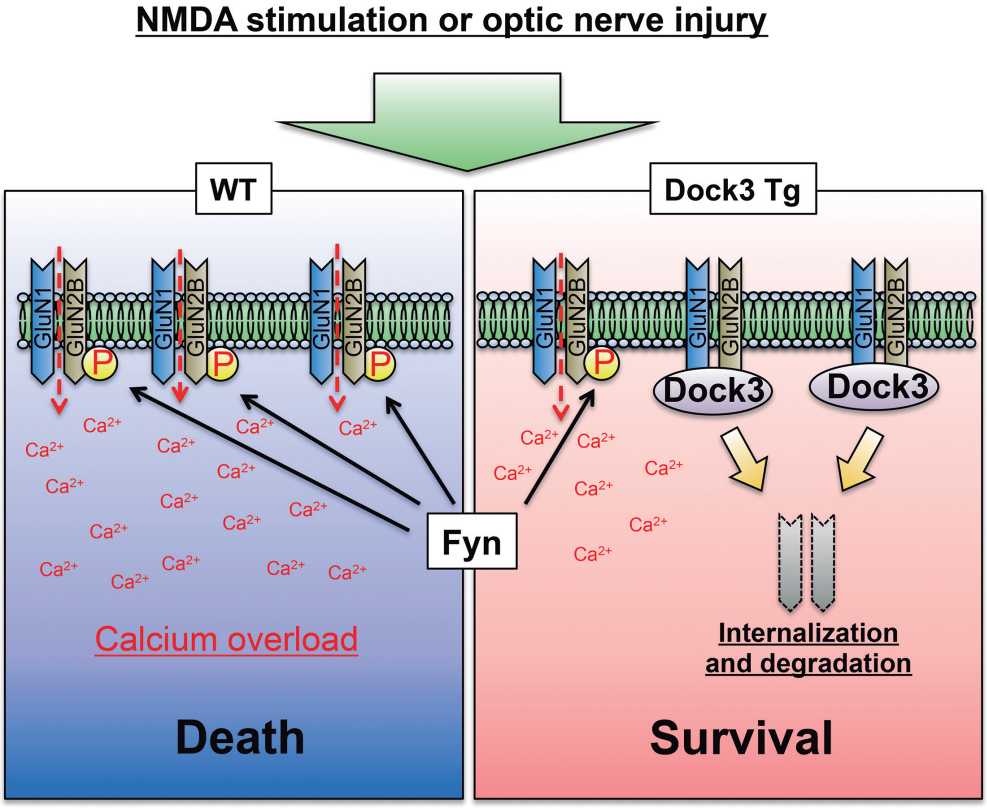

Fig. 2. Proposed mechanisms of Dock3-mediated neuroprotection. Following NMDA stimulation or optic nerve injury, NMDA receptors are activated and Ca^{2+} enters into neurons. In the WT retina, the tyrosine kinase Fyn phosphorylates GluN2B at Tyr1472, which stabilizes the receptor causing excess amount of Ca^{2+} influx that results in retinal ganglion cell (RGC) death. In the Dock3 Tg retina, a large number of Dock3 binds to GluN2B of the NMDA receptor and inhibits Fyn-mediated phosphorylation of Tyr1472, thereby promoting NMDA receptor internalization. This causes reduced Ca^{2+} influx leading to RGC survival.

of the NMDA receptor function is of great interest especially for treatment of neurodegenerative diseases. Targeting the Dock3-GluN2B interaction may not only be beneficial for glaucoma therapy, but also for other neurodegenerative diseases.

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