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Ribonuclease 5 coordinates signals for the regulation of intraocular pressure and inhibits neural apoptosis as a novel multi-functional anti-glaucomatous strategy

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ABSTRACT

Glaucoma is a vision-threatening disorder characterized by progressive death of retinal ganglion cells (RGCs), although little is known about therapeutic milestones. Due to its complex and multifactorial pathogenesis, multipronged therapeutic approach is needed. Angiogenin (ANG), now called ribonuclease (RNase) 5, has been previously known as angiogenic factor and more recently its biologic activity is extended to promoting cell survival via its ribonucleolytic activity. Here, we revealed the defect of ANG in human glaucomatous trabecular meshwork (TM) cells and identified novel multiple functions of ANG as an anti-glaucomatous strategy. ANG was highly expressed in normal eyes and normal TM cells compared to glaucomatous TM cells. ANG induced intraocular pressure (IOP) lowering in rat models of both normal and elevated IOP, and as a possible mechanism, activated Akt-mediated signals for nitric oxide (NO) production, an important regulator of IOP in glaucomatous TM cell. Moreover, we demonstrated ANG-induced production of matrix metalloproteinase (MMP)-1 and -3 and rho-kinase inhibition for TM remodeling. For anti-glaucomatous defense optimization, ANG not only elicited immune-modulative pathways via indolamine 2,3-dioxygenase (IDO) activation in TM cells and suppression of Jurkat T cells, but also rescued neural stem cells (NSCs) from apoptosis induced by glaucomatous stress. These results demonstrate that novel multi-functional effects of ANG may have benefits against glaucoma in ocular tissues.

1. Introduction

Glaucoma is an ocular disorder characterized by progressive death of retinal ganglion cells (RGCs), eventually leading to vision loss if untreated, and is the world's leading cause of the irreversible blindness [1]. As the world population grows and ages, the total number of people affected by glaucoma will continue to increase and is expected to be 80 million including 11.2 million blind by 2020 [2,3].

Primary open-angle glaucoma (POAG), which comprises the majority of primary glaucoma, has three targets: the trabecular meshwork (TM) in the anterior chamber of the eye, RGCs and visual cortex, in an ascending order [4–6]. It has been reported that the visual field defects due to RGC degeneration are directly proportional to TM damage [7]. Although still not fully revealed, IOP increases by malfunctioning TM, as one of the main culprits of visual field defect in glaucoma, may create a mechanical stress that is transmitted posteriorly and damages the RGCs [8]. To date, accordingly, understanding TM cell protection against damage has become a key topic for the treatment and prevention of glaucoma [9]. The various molecular alterations in anterior chamber induce apoptotic TM damage and TM cellularity [10], furthermore increase intraocular pressure (IOP) [11].

Thus, it is needed that targeting diverse pathogenic causes for glaucoma in TM beyond only ameliorating the resultant IOP increase should be emphasized. In the similar context, for example, dorzolamide hydrochloride and timolol maleate, which are the currently famous anti-glaucomatous agents, are recently known to exert protective effect towards oxidative stress in TM as well as to demonstrate well-known IOP lowering effect [12]. Several discoveries indicate that common factors are involved in TM and retinal changes in glaucomatous cascades [13,14] and encourage the hope that targeting TM itself is very important and may also cover the visual field alteration, a main event in glaucoma, at the end.

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Ribonuclease (RNase) 5 which is the 5th member of RNases and is also called angiogenin (ANG), is a 14.4 kDa single-chain polypeptide [15] and it was originally isolated from conditioned medium of cultured human colon adenocarcinoma cells [16]. Now, RNase 5 is known to undergo nuclear translocation and stimulate rRNA transcription to perform its various activities [17]. Recent studies about loss-of-function of ANG in amyotrophic lateral sclerosis [18–22] and Parkinson disease [22,23] demonstrates that RNase 5 is important for normal homeostatic functions. RNase 5 is also known to reprogram protein translation for cell survival under adverse conditions [17,24]. In the field of ophthalmology, Sack et al. reported that ANG is highly concentrated in normal tear fluid pooled overnight with maintaining the corneal avascularity, indicating probable physiologic roles for ANG rather than angiogenesis in normal eyes [25].

ANG has been suggested to be responsible for generating nitric oxide (NO) by activating endothelial NO synthase (eNOS) [26]. eNOS is well-known to be involved in modulating the endothelial permeability as a key enzyme of vascular homeostasis. Interestingly, the vascular balance mechanism between vasoconstriction and vasodilation is also necessary for homeostatic IOP regulation [27]. In a previous study, diverse vascular biomarkers were identified in POAG aqueous humor compared to normal [28] and ubiquitin, as one of them, is known to regulate eNOS activity [29]. Recently, NO is highlighted to contribute to the homeostatic mechanism maintaining aqueous humor outflow and normal IOP as an emerging target for the treatment of glaucoma [30,31]. Furthermore, we have reported the survival effect of NO in corneal fibroblasts under a low range of concentrations, metaphorically called the “double-edged sword” [32]. In addition, ANG can be a candidate survival booster for trabecular meshwork (TM) cells in TM, which is a key area for maintaining IOP, based on their embryonic origin from the neural crest [33] and the recently revealed neuroprotective roles of RNase 5.

As ANG is identified pathologically deficient in several human diseases, we investigated ocular expressions of ANG and further revealed the novel effects of ANG. For anti-glaucomatous ocular optimization, ANG, the synonym of RNase 5, showed multi-functional effects including IOP regulation via activation of an array of signaling pathways, TM cell survival, immune-modulation in TM and anti-apoptosis of neuronal lineage stem cell against glaucomatous damages.

2. Materials and methods

2.1. Study approval

The study protocol and the consent form were approved by the institutional review board of the Chung-Ang University Hospital. All procedures were performed according to the tenets of the Declaration of Helsinki, and informed consent was obtained for the use of cadaveric ocular tissues.

2.2. Reagents and antibodies

The detailed information about reagents and antibodies used in this study are shown in Supplementary Materials and Methods.

2.3. Immunohistochemical staining of cadaveric ocular tissue

The eyes donated for research were pre-fixed in 4% paraformaldehyde solution for 6 h, and cut perpendicular to the cornea, including cornea, limbus, and conjunctiva. The tissue was fixed at room temperature for 6 h. The next serial steps are shown in Supplementary Materials and Methods.

2.4. Animals

All animal experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male Sprague–Dawley rats (8-weeks-of-age; weight, 250–270 g), raised at the Clinic Research Center, Chung-Ang University, College of Medicine, were used.

2.5. Preparation of ocular hypertensive rats as a glaucoma rat model

To test and establish the glaucoma rat model, eight eyes of eight white rats were used in each vortex venous ablation glaucoma model and steroid-induced glaucoma model and the contralateral eyes were served as sham operated control. The information about preparation of both models is shown in Supplementary Materials and Methods.

2.6. Instillation of eye drops in the test rat models

We instilled two drops of ANG (50 μg/mL; 12 eyes) or 50 μg/mL latanoprost, a representative $PGF_{2\alpha}$ analogue (Xalatan™, Pfizer; 12 eyes) or normal saline (12 eyes) in 4 μL/drop in the normal IOP rat and high IOP rat models to compare the ocular hypotensive effects of ANG and the $PGF_{2\alpha}$ analogue. The eye drop agents were administered at 8 am and 8 pm to compare day- and night-time effects, respectively.

2.7. ANG-Cy3 tagging

Purified ANG was labeled with a Cy3 mono-reactive dye (GE Healthcare, Piscataway, NJ, USA) according to the manufacturer's protocol. The Cy3-conjugated ANG (ANG-Cy3) was purified using PD-10 columns (GE Healthcare). Labeling efficiencies were assessed using the Cy3 extinction coefficient at 550 nm and 280 nm. The protein-to-dye ratio was 0.6.

2.8. Administration of ANG-Cy3 eye drops in the rat model

ANG (50 μg/mL) tagged with Cy3 mono-reactive fluorescence was applied to rat eyes every 4 h for 2 days. After the enucleation, the eyeball was cryo-sectioned axially, and frozen biopsy was performed immediately without washing or staining. Then, we observed the distribution of ANG in the eye under fluorescence microscope compared with that of 4′,6-diamidino-2-phenylindole (DAPI) staining.

2.9. Tear collection and aqueous humor extraction in eyes

The detailed information is noted in Supplementary Materials and Methods.

2.10. Primary TM cell

The normal TM tissues were obtained from four post-mortem non-diseased human eyes at the time of corneal transplant and four trabeculectomy specimens from POAG patients were used as glaucomatous TM tissues to prepare the non-transformed primary human normal TM cells (pNTM) and glaucomatous TM cells (pGTM), respectively. The TM tissues were dissected and isolated from eyes as previously described by Wordinger et al. [34]. The detailed processes for culture are shown in Supplementary Materials and Methods. Primary TM cells used for experiments were restricted to 3 to 5 passages.

2.11. Transformed TM cell line and neural stem cell (NSC)

SV40-transformed human TM cell lines derived from normal subjects and patients with glaucoma (NTM5 and GTM3, respectively) were provided by Alcon Laboratories (Fort Worth, TX, USA). The immortalized human NSC clone HB1.F3 was obtained from Prof. S.U. Kim (Chung-Ang University College of Medicine). The detailed processes are shown in Supplementary Materials and Methods.