HHS Public Access

Author manuscript

J Nat Sci. Author manuscript; available in PMC 2015 May 19.

Published in final edited form as:

J Nat Sci. 2015 May 1; 1(5): e103–.

Subtype-dependent Morphological and Functional Degeneration of Retinal Ganglion Cells in Mouse Models of Experimental Glaucoma

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Abstract

In this short review, Puyang and her colleagues compared the results from three laboratories on the dendritic and functional degeneration of retinal ganglion cells (RGCs) in mouse models of experimental glaucoma [1–4]. Acute or chronic ocular hypertension was induced in mice, and different techniques were applied to identify RGC types. The dendritic alternations of RGCs were examined following the induction of ocular hypertension, and their light response properties were characterized by the multi-electrode array (MEA) recording. These studies support the notion that the morphological and functional degeneration of RGCs are subtype-dependent in experimental glaucoma.

Keywords

Retinal Ganglion Cells (RGCs); Experimental Glaucoma; Dendritic Degeneration; Multi-Electrode Array (MEA)

Many studies suggested that subtle changes in dendritic structure and synaptic functions of RGCs precede cell death in mice with experimental glaucoma [5, 6]. Therefore, characterization of morphological and functional degeneration of RGCs at early stages of glaucoma may open a time window for treatment to prevent subsequent vision loss. There are more than 20 distinct types of RGCs in mouse [7–11], which makes it challenging to profile how each RGC type degenerates with glaucoma progression. Combined mouse genetics, molecular biology, and physiology, studies began to reveal the effects of glaucomatous insult on different RGC types. In this review, we compared the results from

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Conflict of interest: No conflicts declared.

three laboratories on the subtype-dependent degeneration of RGCs in mice with experimental glaucoma [1–4].

Because elevated intraocular pressure (IOP) is an important risk factor for the development of glaucoma, ocular hypertension is often induced in mice to mimic human high-tension glaucoma (Table 1). Injection of polystyrene microbeads into the anterior chamber, which occludes the aqueous outflow, induces acute IOP elevation [3, 12]. Repeated injections can be applied in order to maintain long-term IOP elevation [2, 12]. By comparison, laser illumination was applied to the corneal limbus to photocoagulate the aqueous outflow, which in turn induced IOP elevation for more than 2 months [1, 4, 13]. We further combined microbead injection and laser illumination into one procedure to achieve IOP elevation up to 5 months [4].

Different methods/techniques were applied to characterize RGC types. El-Danaf and his colleagues used two transgenic mouse lines which had OFF-α RGCs and direction-selective RGCs (DSGCs) labeled, respectively [3]. DSGCs filled with Alexa Fluor 555 hydrazide were separated into two groups: ON- and ON-OFF DSGCs [3]. Thy-1-YFP mice were also used which had a small number of RGCs labeled [1, 2, 9, 14]. Based on the signature laminar pattern of alpha-like RGCs, they were classified into ON-sustained (A-Type ON-S), OFF-sustained (A-Type OFF-S), and OFF-transient (A-Type OFF-T) subtypes [2, 15]. We classified RGCs into ON, OFF, and ON-OFF types also based on the lamination pattern of dendrites in the inner plexiform layer (IPL) [1]. In addition, immunostaining with antibodies against melanopsin and SMI-32 were performed to label specific RGC types [1, 3, 9, 16].

As early as one week post IOP elevation, one of the major morphological changes is the dendritic alternation in the OFF sublaminar of the IPL [3]. At 2–4 weeks post IOP elevation, Della Santina and his colleagues showed that OFF-transient RGCs exhibited decreased dendritic coverage, dendritic length, and number of dendrites [2]. At 6–8 weeks post IOP elevation, we found that the dendritic coverage of mono-laminated ON but not bi-laminated ON-OFF cells decreased [1]. We further showed that the dendritic branching of a subtype of ON cells, the SMI-32-positive ON cells, was significantly reduced [1]. All these studies suggested that deterioration of dendritic morphology was detected at the very early stage of glaucoma and that the dendritic trees of RGCs continued to degenerate in a subtype-dependent manner.

Given that the dendritic structure of an RGC determines its function in visual information processing, the light response properties of an RGC may be altered correspondingly. Studies from two laboratories using the MEA recording demonstrated that the functional degeneration of RGCs is also subtype-dependent (Table 2) [2, 4]. Wong laboratory classified RGCs into ON and OFF cells based on their responses to a square-wave stimulus, then subgrouped them to sustained or transient types [2]. Spike-triggered average (STA) analysis was applied to characterize a neuron’s receptive field (RF) properties [2]. By contrast, we applied the non-centered spike-triggered covariance (STC-NC) analysis to classify RGCs into ON, OFF, and ON-OFF three types [4, 17]. In both studies, the activity strength of RGCs were investigated [1, 2, 4]. Wong laboratory reported that, at 4 weeks post IOP elevation, the spontaneous activities and maximal spike rate of the light responses

decreased for ON-sustained, OFF-sustained and OFF -transient RGCs, but not for ON-transient cells [2]. Our data showed that the average firing rates of all visually-responsive RGCs decreased after five weeks of IOP elevation [4]. Wong laboratory demonstrated that the RF sizes of OFF-transient RGCs significantly decreased [2], similar to our findings [4]. In addition, we showed that the RF sizes of ON RGCs, but not ON-OFF RGCs, were reduced [4]. Interestingly, the large ON and OFF RGCs were very sensitive to the hypertensive insult [4], consistent with the morphological studies in monkey and cat with experimental glaucoma [18, 19]. Together our studies as well as studies from other groups support the notion that the dendritic and functional degeneration of RGCs are subtype-dependent in experimental glaucoma.

Many important questions remain unanswered. For example, the dendritic degeneration of an RGC subtype does not always correlate with its functional changes as listed in Tables 1 and 2. Moreover, how do different RGC subtypes respond differently to the hypertensive insult? Some studies suggested that the different susceptibility to pressure in RGCs could be due to the differential expression of transient receptor potential vanilloid (TRPV) channels [20–22]. The vasculature structure may also contribute to the dendritic degeneration of RGCs. For example, the OFF sublamina, unlike the ON sublamina of the IPL, is highly vascularized with capillaries, which makes the OFF sublamina more vulnerable than the ON sublamina at the early stage of IOP elevation [3]. Early signs of RGC damage have also been detected at the axon terminals [23–25]. Selective damage of axons may also contribute to the subtype-dependent RGC loss [4, 22–24]. Finally, during development, RGCs mature also in a subtype-dependent manner [9, 26, 27]. Misregulation of RGC structure and synaptic function during development leads to devastating vision losses such as in the childhood glaucoma. A new mouse model in which IOP was elevated dramatically during the first month after birth provides an opportunity to examine how development and function of RGCs are misregulated in the diseased condition [28]. More studies are needed to better understand the subtype-dependent RGC degeneration and its underlying mechanisms, which will add important insights on how to protect RGCs and vision in glaucoma.

Acknowledgments

This work was supported by the National Institutes of Health (NIH) grant R01EY019034 (to X.L.), the Dr. Douglas H. Johnson Award for Glaucoma Research from BrightFocus Foundation (to X.L.), Northwestern Memorial Foundation/Brinson Foundation (to X.L.), and the unrestricted RPB grant to the Department of Ophthalmology, Northwestern University.

This manuscript mainly reviewed the following 4 papers

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J Nat Sci. Author manuscript; available in PMC 2015 May 19.

Table 1

Morphological Degeneration of RGCs in Mouse Models of Experimental Glaucoma.

References	Mouse model	IOP elevation	Duration of IOP elevation	Dendritic field size	Dendritic branching pattern	
					Parameters measured	Changes
El-Danaf and Huberman, 2014	Microbead injection	mild, acute	1 week	α OFF-transient ↓ ON, ON-OFF DSGC and M1 ipRGC ↔	dendritic complexity, number and total length of dendrites	OFF sublaminar ↓ ON sublaminar ↔
Della Santina, et al., 2013	Microbead injections	modest, chronic	2 – 4 weeks	OFF-transient ↓ ON and OFF-sustained ↔ ON transient: not analyzed	dendritic complexity, number and total length of dendrites	OFF-transient ↓ ON and OFF-sustained ↔
Feng, et al., 2013	Laser illumination	modest, chronic	6 – 8 weeks	ON↓ ON-OFF ↔ OFF: not analyzed.	total length of dendrites	SMI-32-positive ON ↓

Note 1. DSGC: direction-selective ganglion cell; ipRGC: intrinsically photosensitive retinal ganglion cells.

Note 2. ↓ significant decrease; ↔ No significant change (same for Table 2).

Table 2

Functional Degeneration of RGCs in Experimental Glaucoma.

References	Duration of IOP elevation	Subtypes	Spontaneous Activity	Spike Rate of the Light Responses	Receptive Field (RF) Size
Della Santina, et al., 2013	2 – 4 weeks	ON-sustained	↓	↓	↔
		ON-transient	↔	↔	↔
		OFF-sustained	↓	↓	↔
		OFF-transient	↓	↓	↓
Chen, et al., 2015	5 – 6 weeks	ON		↓	↓
		OFF			↓
		ON-OFF			↔