Characterization of retinal ganglion cell loss in a mouse model with elevated IOP
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INTRODUCTION
Progressive retinal ganglion cell (RGC) degeneration is the best recognized pathology of glaucoma and the extent of RGC degeneration in glaucoma is closely correlated with the extent of intraocular pressure (IOP) elevation. Recent studies employed transgenic mouse lines, in which fluorescent protein is expressed by RGCs, to monitor the RGC degeneration in vivo. However, some studies demonstrated that the expression of fluorescent protein in RGCs could be affected by elevated IOP in glaucomatous animal models before RGC death. Therefore, it is still uncertain whether in vivo monitoring the fluorescent protein-expressing RGCs in glaucomatous animal models could provide an index of RGC degeneration in vivo. In this study, we characterized the RGC loss in a mouse model with elevated IOP using an in vivo imaging approach and compared the results with in vitro study.

METHODS
Animals: WT mice and Thy1-CFP mice aged 1 to 6 months were used in this study.
Elevated IOP model: Elevated IOP model was generated by intracameral injection of microbeads into the anterior chamber.
IOP measurement: A week after IOP was measured 3 consecutive days before and after the day of microbead injection using TonopenIllion.jpg.
In vivo imaging of RGCs: In vivo imaging of Thy1-CFP mice with elevated IOP was performed using confocal scanning laser scanning of RGCs in fixed retina 1, 2, 3, and 4 weeks after IOP elevation.

RESULTS
Establishing the elevated IOP model by injecting fluorescent microbeads into the anterior chamber
A. In vivo image of the fluorescent microbeads located at the posterior region of the anterior segment toward the area of aqueous outflow (arrows). 3 weeks after the intracameral injection, B. the cross section of the anterior segment of any eye 6 weeks after the microbead intracameral injection showing that microbeads are apparent in the iris-corneal angle and closer to the point of aqueous outflow (arrows).

Progressive loss of Brn3b-expressing RGCs in eyes with elevated IOP
RGC density was examined in control eyes and eyes with intracameral injection of microbeads 1, 2, 3, and 4 weeks after the elevation of IOP. RGCs were immunostained using anti-Brn3b antibody. A. A confocal imaging of DAPI (blue) and anti-Brn3b (red) labeling of retina from an eye 4 weeks after microbeads injection with elevated IOP. B. A confocal imaging of DAPI (blue) and anti-Brn3b (red) labeling of retina from an eye 4 weeks after saline injection. C. A confocal imaging of DAPI (blue) and anti-Brn3b (red) labeling of retina from an eye 4 weeks after microbeads injection with elevated IOP. D. A confocal imaging of DAPI (blue) and anti-Brn3b (red) labeling of retina from an eye 4 weeks after saline injection.

CONCLUSIONS
1. Anterior chamber injection of microbeads effectively induced IOP elevation in mouse eyes.
2. A progressive RGC death is observed in mouse eyes with elevated IOP using in vivo immunohistochemical staining of RGCs.
3. In vivo confocal scanning laser microscope imaging of transgenic mice with CFP expressed in RGCs provides an effective and noninvasive approach to monitor the progress of RGC damage.