EFFECT OF BAMBI EXPRESSION ON THE ECM OF TRABECULAR MESHWORK AND INTRAOCULAR PRESSURE IN MICE

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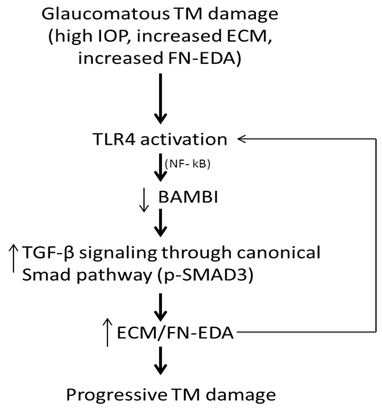
INTRODUCTION

Elevated intraocular pressure (IOP) is one of the primary risk factors in the development of glaucoma. The trabecular meshwork (TM) is involved in the outflow of aqueous humor and IOP regulation. TGF-β signaling pathways in the extracellular matrix (ECM) of the TM have been extensively studied. Evidence has implicated toll-like receptor 4 (TLR4) in the regulation of ECM and fibrogenesis in liver, kidney, lung and skin. In addition, we have recently identified a TGFβ2-TLR4 signaling crosstalk in the TM. BMP and the activin membrane-bound inhibitor (BAMBI), is known to enhance TGFβ signaling leading to increased ECM production. BAMBI downregulation by TLR4 is regulated by the MyD88-NFκB-dependent pathway. Here we test the effect of knockdown of BAMBI on intraocular and ECM protein expression in the TM of B6;129S1-*Bambi*^{tm1Jian}/J floxed mice.



METHODS

Eyes were fixed in 4% paraformaldehyde for 24 hours, processed and embedded in paraffin. 5-µm sections were cut and sections were transferred to glass slides. Paraffin sections were dewaxed 2 times in xylene, 100% ethanol, and 95% ethanol for 2 minutes each. Slides were washed 3 times in PBS for 5 minutes each, blocked with SuperBlock for 1 hour at room temperature. Sections were labeled with primary antibody (2 hours at room temperature using BAMBI and Fibronectin conjugated antibody) and labeled with secondary antibody (2 hours at room temperature using anti-rabbit 488 and anti-mouse 594 conjugated antibody). After 3 washes in PBS, the sections were mounted with ProLong™ Gold Antifade Mountant with DAPI and viewed with fluorescence microscopy.

RESULTS

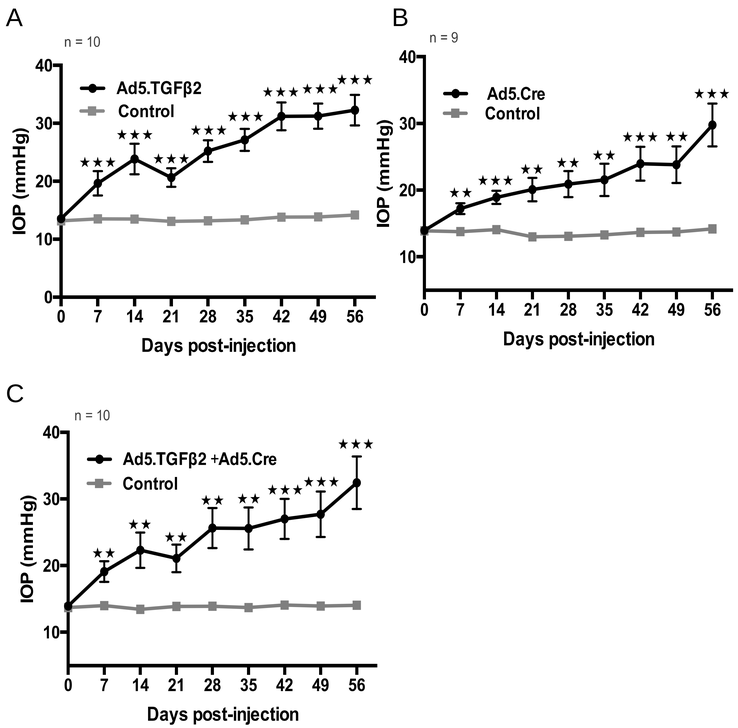
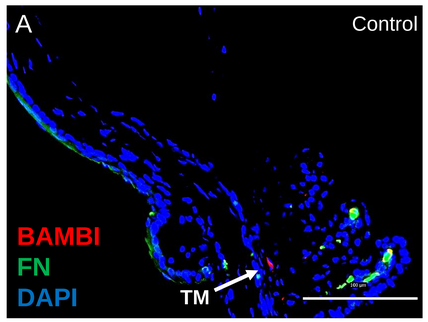


Figure 1. Effects of Ad5.hTGFβ2$^{226/228}$ and Ad5.Cre on IOP in mouse eyes. (A) Mice were injected intravitreally with Ad5.Cre, Ad5.hTGFβ2$^{226/228}$, or Ad5.hTGFβ2$^{226/228}$ + Ad5.Cre. Day of injection was designated as day 0. In both studies, the contralateral eye of each mouse was uninjected and served as a paired control. (A, B, C) Injection with either Ad5.Cre, Ad5.TGFβ2, or Ad5.TGFβ2 + Ad5.Cre each induced ocular hypertension starting at day 7 post-injection and maintained significant IOP elevation throughout the 56 day time course compared to uninjected control eyes ($p<0.01$, days 7-56). (A) Ad5.TGFβ2 (32.3 +/- 2.6 mm Hg) had significant IOP elevation at 56 days post-injection compared to 14.2 +/- 0.3 mmHg in uninjected control eyes ($p<0.001$). (B) At day 56 post-injection IOP increased to 29.8 +/- 3.2 mmHg in Ad5.Cre injected eyes compared to 14.2 +/- 0.3 mmHg in contralateral uninjected eyes ($p<0.001$). (C) Ad5.TGFβ2 + Ad5.Cre (32.4 +/- 3.9 mmHg) also had significant IOP elevation at 56 days post-injection compared to uninjected control eyes (14.1 +/- 0.3 mmHg), $p<0.001$. These data performed by Humberto Hernandez.



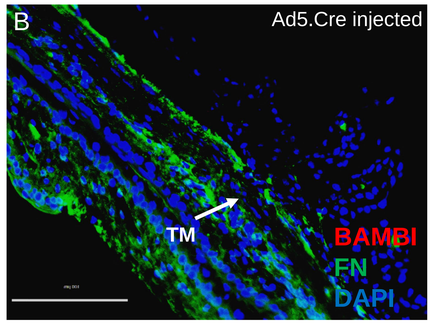


Figure 2. Ad5.Cre knockdown of BAMBI in the mouse TM.(A) Uninjected control eyes for BAMBI protein expression (red) in the TM. (B) Ad5.Cre injected eyes demonstrated no BAMBI expression. Ad5.Cre injected eyes also had an increase in fibronectin expression (green) compared to uninjected control eyes. N=3

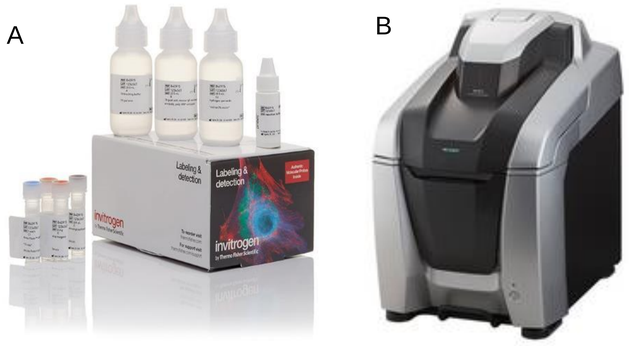


Figure 3. Pictorial representation of the (A) Alexa Fluor 594 bright, red-fluorescent dye that can be excited using the 561 nm or 594 nm laser lines and (B) inverted BZ-X710 fluorescence phase contrast microscope with bright field, fluorescence (wide-field/sectioning), Phase contrast (PhL, Ph1, Ph2), and oblique illumination in the infinite optical system Nikon CFI 60 Series.

CONCLUSION

These studies identify BAMBI as an important molecule in ECM production and IOP regulation in the TM. These data also further support our hypothesis of a TGFβ2 – TLR4 crosstalk as a novel pathway involved in ECM regulation in the TM and ocular hypertension. These data provide potential new targets to lower IOP and further explain the mechanisms involved in the development of glaucomatous TM damage.

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