The effect of CRMP5 in an experimental in vivo and in vitro model of glaucoma on neuroprotection and neuroregeneration of retinal ganglion cells in vitro

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Purpose: Collapsin-response-mediator-protein 5 (CRMP5) is supposed to play an important role in axon formation of neurites and growth cone guidance. Purpose of this study was to analyze the role of CRMP5 in an animal model of glaucoma in vivo and to test its potential neuroprotective and neuroregenerative effects on retinal ganglion cells (RGCs) and their axons in retinal explants cultured from Sprague Dawley rats in vitro.

Methods: Elevated intraocular pressure (IOP) was induced in adult Sprague-Dawley (SD) rats by cauterization of episcleral veins. Proteomic changes of CRMP5 within the retina were analyzed via label-free mass spectrometry. In vitro, retinal explants from adult SD rats were cultured under elevated pressure (60 mmHg) within a high pressure incubation chamber over two days with and without addition of 200 µg/l CRMP5. An antibody against Protein Kinase B (PKB) was added to examine a potential interaction with CRMP5. RGC count was performed in retinal flat mounts. Following optic nerve crush and lens injury, which was performed 3 days prior to explantation, retinal explants were cultured under regenerative conditions with and without addition of CRMP5 over 7 days. The number and length of axons were determined and mean values were built and compared. To prove possible intracellular pathways CRMP5 and PKB were detected via microarray and immunohistochemistry.

Results: CRMP5 was downregulated 3-fold due to chronically elevated IOP in animals (fig. 1A). Addition of CRMP5 to retinal culture could significantly increase the amount of RGCs in vitro (fig. 1E). The survival rate was investigated in retinal flatmounts stained against BRN3A (fig. 1B-D). Furthermore CRMP5 could increase and elongate axonal outgrowth after optic nerve crush and lens injury in retinal explants (fig. 3) illustrated through ß-III-Tubulin-staining (fig. 2). Especially the amount of neurites longer than 400 µm significantly increased after addition of CRMP5 (fig. 4). CRMP5 as well as PKB were detected higher in the experimental than in the control group (fig. 5 and 6).

Neuroprotective effects of CRMP5 on retinal ganglion cells

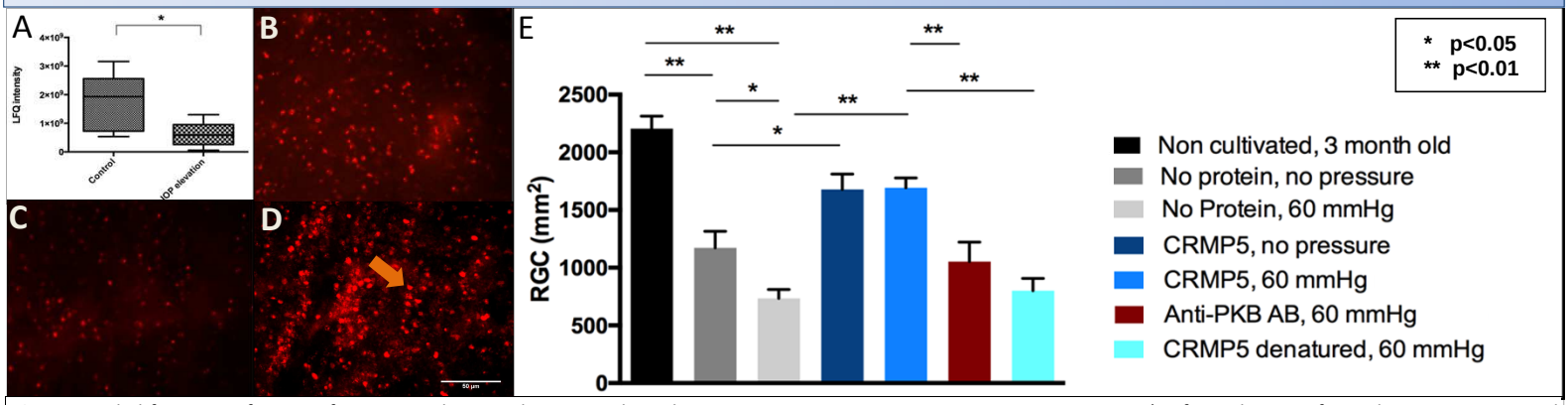

Figure 1: Label free quantification of CRMP5 in the retinal proteom through mass spectrometry (±SD, n=14, parametric t-test; A); After cultivation for 48 h RGCs were stained against BRN3A. RGCs were cultered e.g. without protein and without pressure (n=6; B), without protein and with pressure of 60 mmHg (n=6; C) and with 200 µg/l CRMP5 and with pressure of 60 mmHg . The arrow points at a RGC (n=6; D). Mean comparison of RGC quantification after cultivation (±SEM, n=42, anova-test; E).

Neuroregenerative effect of CRMP5 on retinal ganglion cells

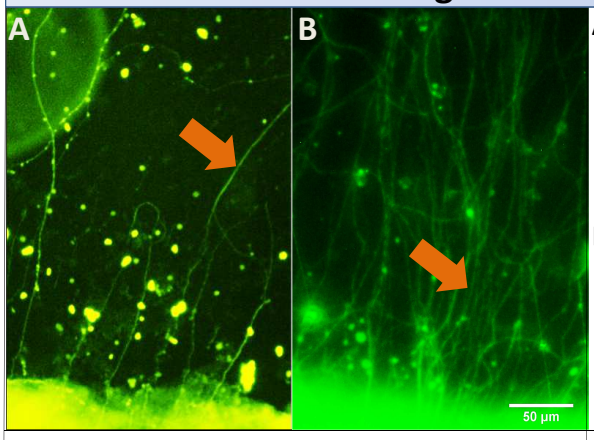

Figure 2: ß-III-Tubulin-staining of outgrowing neurites. Retinal flatmounts were cultured without (n=5; A) and with (n=5; B) CRMP5. The arrows point at outgrowing neurites.

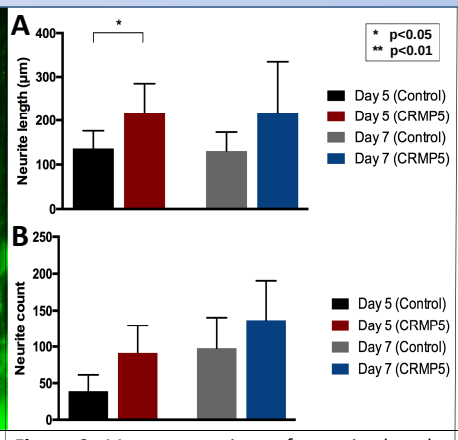

Figure 3: Mean comparison of neurite lengths (±SD, n=5, parametric t-test; A) and the amount of neurites (±SEM, n=5, parametric t-test; B)

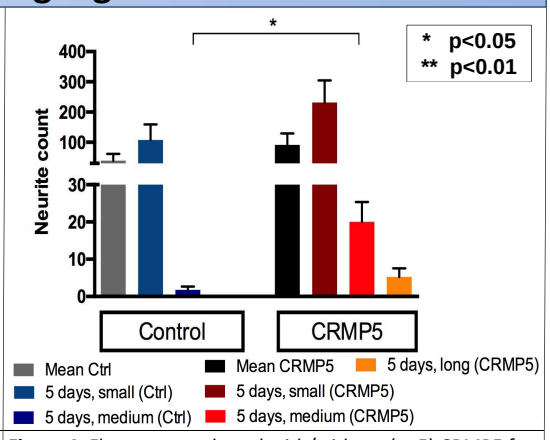

Figure 4: Flatmounts cultured with/without (n=5) CRMP5 for 5 days. Quantification of development into 3length (<400µm; 400-800µm and >800µm) ; (±SEM, parametric t-test).

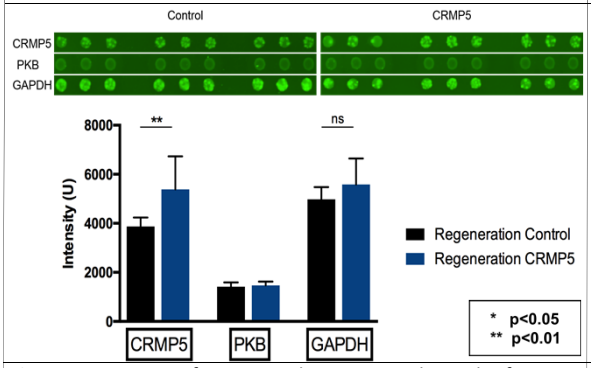

Figure 5: Detection of CRMP5 and PKB in retinal samples from the regeneration study through antibody-based microarray (±SD, n=8, parametric t-test)

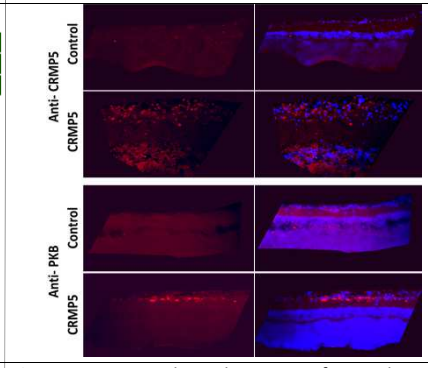

Figure 6: Immunohistochemistry of retinal cross sections cultured without and with CRMP5, stained against CRMP5 and PKB

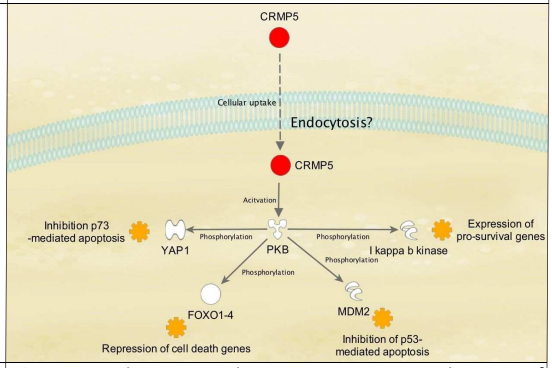

Figure 7: The potential neuroprotective mechanism of CRMP5. Activation of PKB might lead to various intracellular anti-apoptotic effects (IPA Ingenuity Pathway Analysis).

Conclusion: CRMP5 seems to play an important pathophysiological role in an animal model of glaucoma and exerts both neuroprotective and neuroregenerative effects in vitro. Additionally CRMP5 is known to be expressed almost only in the postnatal CNS. Substitution of CRMP5 could enhance the RGC survival tremendously. Furthermore it enabled RGCs to a higher regeneration rate and to an accelerated neurite outgrowth. This effect could be possibly mediated by an activation of PKB.