The transcription factor Runx1 as a novel mediator of astrocyte reactivity in the optic nerve

Song Li and Tatjana C. Jakobs

Massachusetts Eye and Ear infirmary, Harvard Medical School, Department of Ophthalmology, Boston, United States

Purpose:

Astrocyte reactivity is a complex process that involves changes in gene expression and morphological remodeling. The optic nerve head contains a focal concentration of astrocytes that ensheath the unmyelinated axons of retinal ganglion cells and form their direct environment. We studied the regulation of astrocyte reactivity by the transcription factor Runx1.

Background:

Injury to the CNS, including the optic nerve leads to astrocyte reactivity. Originally considered a pathological response, it has recently become clear that the consequences of astrocyte reactivity depend on the nature of the injury and the spatial and temporal distance to the injury site. We have shown that in several mouse models of optic nerve damage (the microbead model of ocular hypertension, a short-term elevation of intraocular pressure, and optic nerve crush) astrocyte reactivity is protective and interfering with it leads to a worse outcome for retinal ganglion cell survival and visual function (Sun, 2017). However, the mechanisms by which astrocytes protect retinal ganglion cell health in early glaucoma are not well understood. We compared several gene expression studies of reactive astrocytes in the optic nerve and other parts of the brain from the literature and our own laboratory to find genes and pathways that are common to most or all of these models and observed that the transcription factor Runx1 is up-regulated in the DBA/2J model of glaucoma (Howell, 2011), optic nerve crush (Qu, 2013), middle cerebral artery occlusion (Zamanian, 2012) and the microbead occlusion model of ocular hypertension (unpublished data). Therefore, we investigated the role of the transcription factor Runx1 in astrocyte reactivity.

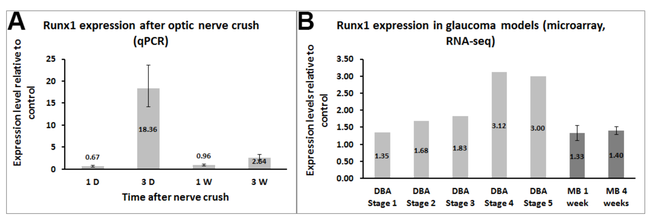


Figure 1; (A) Runx1 is significantly up-regulated 3 days after optic nerve crush. (B) Gene expression screening in the genetic mouse model of glaucoma DBA/2J shows that Runx1 expression is up-regulated in all stages of glaufrom stage 1 (no morphological signs of ganglion cell damage) to 5 (severe damage with almost complete loss of ganglion cells) compared to non-glaucomatous DBA-Gpnmbcoma. Light grey bars represent expression levels + optic nerves. Data from Howell, 2011. Dark grey bars represent Runx1 expression levels in the optic nerve 1 and 4 weeks after microbead injection compared to uninjured controls (our RNA-seq data).

Methods

Astrocytes were isolated from the optic nerve of newborn mice and grown in DMEM/F12. The cells were treated with the Runx1 inhibitor Ro5-3335 (25 µM for 24 h) or the E2f1 inhibitor HLM006474 (40 µM for 24 h). The E2f1 inhibitor was included because we found that E2f1 was also highly up-regulated in reactive astrocytes and may cooperate with Runx1 in regulating gene expression. RNA was extracted and sequenced on a Illumina NovaSeq 600 system. 150 bp pair end reads were generated, aligned with the mouse reference genome, and differentially expressed genes were identified using the R statistical software package. Differentially expressed genes were defined as log2-fold change of >0.58 and a false-discovery rate adjusted p<0.05. Pathway analysis was performed with Ingenuity Pathway Analysis (Qiagen).

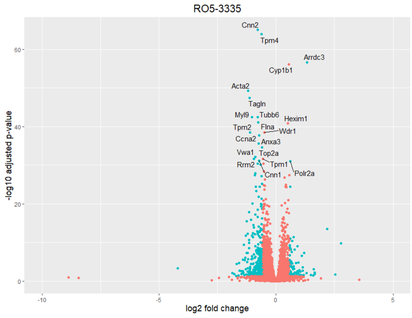


Figure 2. Volcano plot of the RNA-seq dataset in astrocytes treated withRo5-3335 (Runx1 inhibitor). 397 genes were considered differentially regulated.

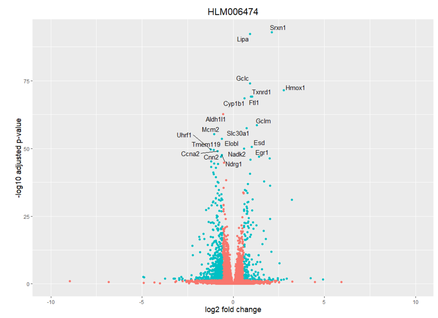


Figure 3. Volcano plot of the RNA-seq dataset in astrocytes treated with HLM006474 (E2f1 inhibitor). 856 genes were considered differentially regulated.

Identification of candidate genes:

Genes in the Ro5-3335 dataset were compared to genes differentially expressed in reactive astrocytes in vivo to reduce the number of potential candidates and rule out genes that may not be related to astrocyte reactivity.

Gene ID	
ADAM12	ADAM metallopeptidase domain 12
ANGPTL4	angiopoietin like 4
CDT1	chromatin licensing and DNA replication factor 1
CH25H	cholesterol 25-hydroxylase
F2RL1	F2R like trypsin receptor 1
IGFBP3	insulin like growth factor binding protein 3
MCM2	minichromosome maintenance complex component 2
MCM6	minichromosome maintenance complex component 6
MMP3	matrix metallopeptidase 3
POLE2	DNA polymerase epsilon 2, accessory subunit
RRM2	ribonucleotide reductase regulatory subunit M2
SLCO2A1	solute carrier organic anion transporter family member 2A1
SPP1	secreted phosphoprotein 1
TNC	tenascin C
TNFAIP2	TNF alpha induced protein 2
TUBB6	tubulin beta 6 class V
UHRF1	ubiquitin like with PHD and ring finger domains 1

Figure 4. Runx1-responsive genes that are differentially regulated in all models of astrocyte reactivity. CDT1, CH25H, F2RL1, MCM2, MCM6, POLE2, SLO2A1, SPP1, and UHRF1 are also regulated by E2f1.

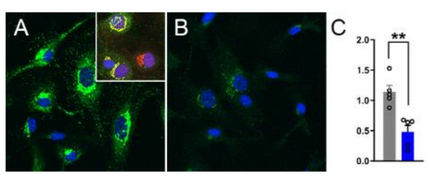


Figure 5. (A) Spp1 (green) is expressed in cultured astrocytes. Inset in A shows co-expression with Runx1 (red). Nuclear counterstain (blue). (B) Treatment with Ro5-3335 leads to pronounced down-regulation of Spp1. (C) Relative mRNA expression levels of Spp1 in untreated (grey bar) and Ro5-3335-treated astrocytes (blue bar).

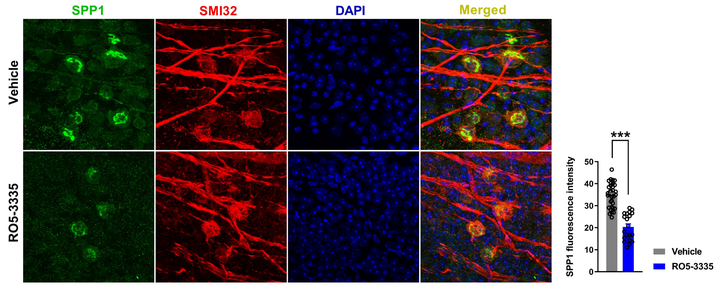
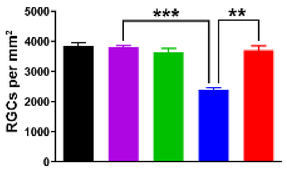


Figure 6. Spp1 is expressed in a type of retinal ganglion cell (alpha cells). We tested whether Runx1 also regulates Spp1 in these cells. Ro5-3335 was injected intravitreally in young adult C57bl/6 mice and the expression of Spp1 (green) was quantified by measuring fluorescence intensity of the immunolabeling. Counterstaining with the alpha ganglion cell marker neurofilament H (SMI32) is shown in red.

Figure 7. Pretreatment with Spp1 overexpression in the retina using AAV2 protects retinal ganglion cells from degeneration in the microbead occlusion model of glaucoma. Black bar, naïve retina; purple, AAV-GFP (control vector) without high IOP; green, AAV-Spp1 without high IOP; blue, AAV-GFP followed by microbead injection; red, AAV-Spp1 followed by microbead injection. No ganglion cell loss was observed after pretreatment with AAV-Spp1. ***, p<0.01, ***, p<0.001.



Conclusions:

About 400 genes are differentially regulated by Runx1 in isolated astrocytes from the optic nerve. Runx1-responsive genes in astrocytes fall into 4 main categories: 1. genes involved in tissue remodeling (metalloproteases, laminins); 2. genes involved in cell cycle progression, chromosome replication, gene transcription, and DNA synthesis; 3. secreted signaling molecules, cytokines, and growth factors (e.g. Spp1, Angptl3, Igfbp3); 3. genes related to prostaglandin signaling; and 4. others (such as components of the cytoskeleton, enzymes). At least one of the Runx1-responsive genes (Spp1) shows neuroprotective activity.

Acknowledgements:

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