Genetic disection of p27-mediated reactive gliosis

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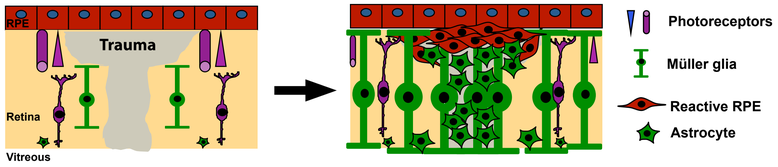
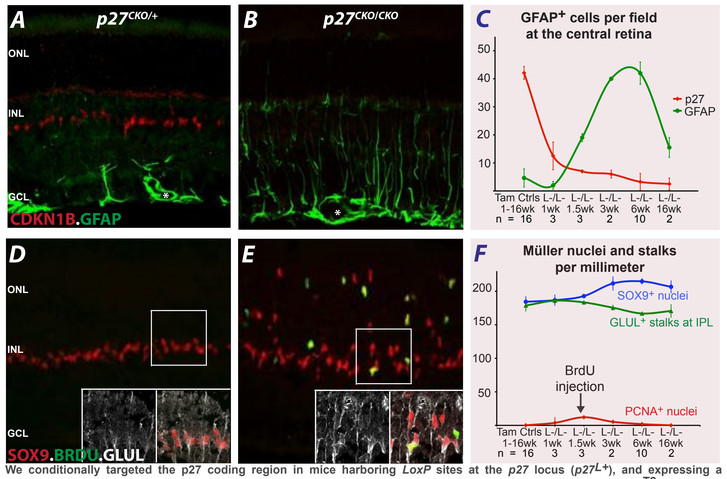
Retinal trauma can activate Müller glia to enlarge, migrate, proliferate, and form scars

Virtually any challenge to adult mammalian CNS activates glia to undergo widespread molecular and cellular transformations, including hypertrophy, proliferation, migration and cytokine release. "Reactive gliosis" contributes to tissue repair but also to dysfunction, scar formation, abberant neural rewiring, and vascular remodeling.



Inducible p27 inactivation in adult retina recapitulates proliferative reactive gliosis

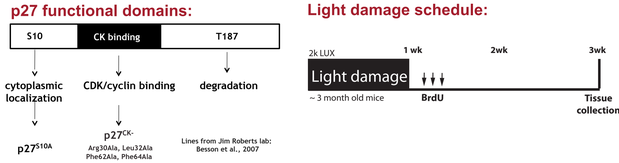
A good candidate to target is the cell cycle inhibitor p27KIP1 (encoded by *Cdkn1b*). p27 downregulation correlates with early stages of glial reactivity and germline p27-deficiency results in a constitutive form of reactive gliosis. We have found out that conditional inactivation of *p27* in adult mice results in upregulation of the intermediate filament GFAP (*A-C*), nuclear migration (*D*, *E*), and cell cycle entry (*D-F*) [Vázquez-Chona, submitted].

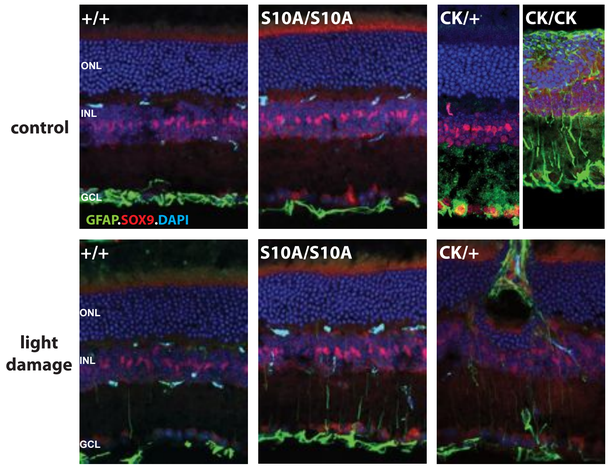


We conditionally targeted the p27 coding region in mice harboring *LoxP* sites at the *p27* locus ($p27^{L+}$), and expressing a tamoxifen-regulated Cre recombinase under the control of the chimeric chicken beta-actin promoter, CAGG::CreER^{T2}.

Loss of p27 binding to cyclin/CDKs increases Müller glial migration and GFAP upregulation

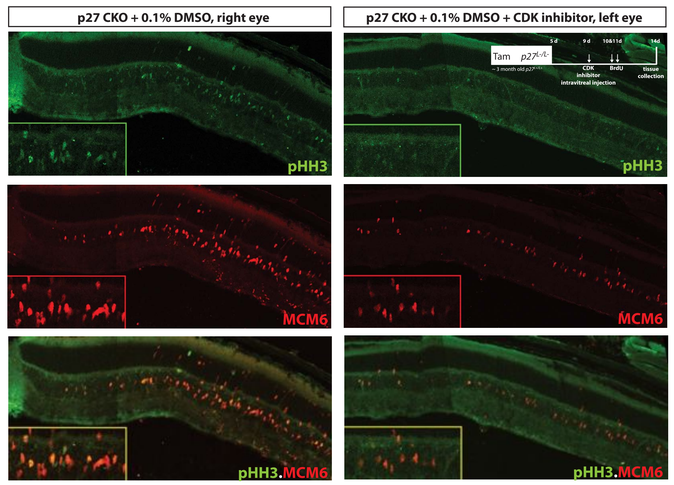
Enhanced phosphorylation on Ser10 correlates with p27 cytoplasmic localization and proliferation in tumors and reactive glia (Besson et al., 2006; Kase et al., 2006). Here, we tested whether the S10 domain or the cyclin/CDK domain mediates Müller glial reactivity using p27 knock-in mutations. We found that the CK- mutation and not the S10 mutation altered the response of Müller glia after light damage.





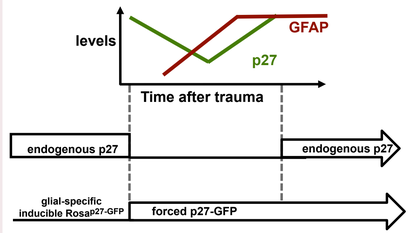
Pharmacological CDK inhibitors decrease Müller glial cell-cycle entry & nuclear migration in the p27 CKO

Pharmacological CDK inhibitors were injected into the eyes of p27 CKO mice. Our results are consistent with published data suggesting that CDK inhibitors decrease glial reactivity as well as enhance neuroprotection.

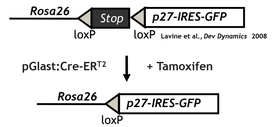


Forced p27 expression decreases OLM breakdown after retinal detachment

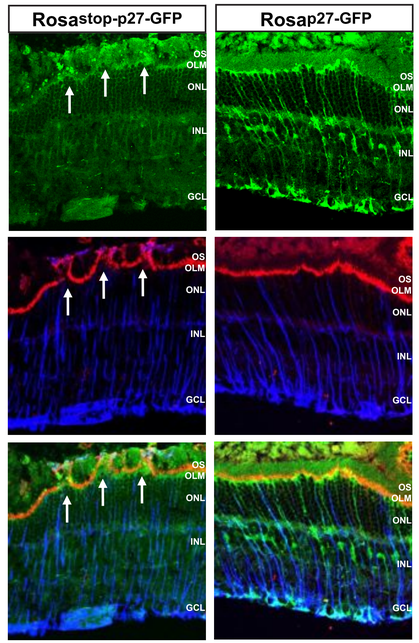
p27 levels become donwregulated in many CNS trauma models, including retinal detachments or tears:



Genetic model of forced p27 expression:



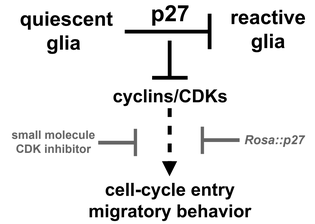
Experimental design and chase:



Effect of forcing p27 on components of glial reactivity in various models

Experimental model	N	GFAP upregulation	proliferation	branch extension	nuclear migration
detachment	4	no effect	reduced	reduced	reduced
light damage	7	no effect	n/a	n/a	n/a
increased intraocular pressure	4	no effect	n/a	n/a	n/a
adult germline $p27^{-/-}$	6	no effect	n/a	n/a	no effect

Model: p27 mediates negative regulation Müller glial reactivity by regulating cyclin-CDK complexes



Our genetic models suggest that p27 is a negative regulator of Müller glial reactivity. Conditional inactivation and forced expression of p27 as well as the loss of p27 binding to cyclins/CDKs suggest that p27 activity is most closely associated with glial migratory behavior and cell-cycle entry; whereas, glial hypertrophy appears to be a secondary event. We are currently optimizing the delivery and therapeutic window of pharmacological CDK inhibitors to rescue the down-regulation of p27 during trauma.

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