Epigenetic control of pathological vascular remodeling: Role of smooth muscle-derived AdvSca1-SM progenitor cell induction of HDAC9-Brg1

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Background

- Atherosclerosis and restenosis induce neointimal formation and pathologic vascular remodeling marked by inflammation and fibrosis. Classically overlooked, the adventitial layer of the blood vessel is the site of dynamic processes that influence vascular physiology.
- Using a vascular smooth muscle (SMC)-specific fate-mapping approach, the Weiser-Evans lab discovered a unique subpopulation of adventitial Sca1$^+$ multipotent progenitor cells that originate from reprogrammed SMCs and are major contributors to vascular adventitial remodeling (**termed AdvSca1-SM cells**)[1].
- Recently, the epigenetic regulators Brg1 and HDAC9 were shown to form a complex to repress SMC gene expression and the mature SMC phenotype[2,3].
- Using RNA-Seq approaches, we found that AdvSca1-SM cells downregulate stemness-related genes (**Figure 1A**) and upregulate Brg1 and HDAC9 in response to vascular injury (**Figure 1B**).

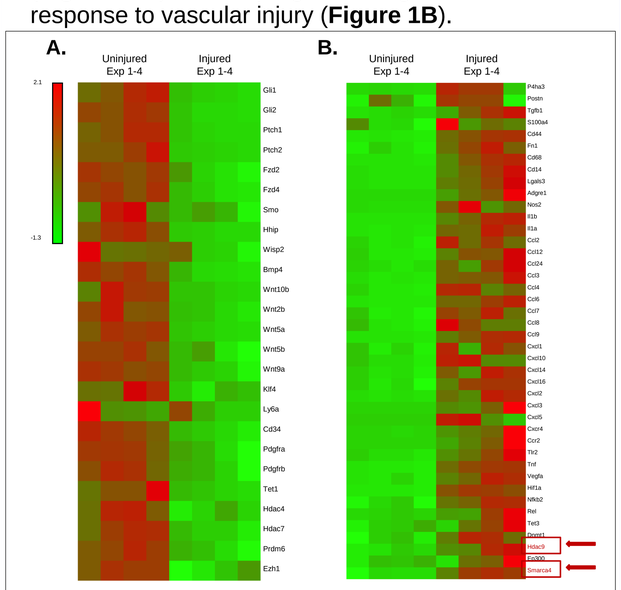


Figure 1. Unbiased RNAseq shows loss of stemness-related genes and upregulation of Brg1 and HDAC9 in AdvSca1-SM Cells in response to injury.
SMC reporter mice underwent CA ligation for 3d timepoint. AdvSca1-SM cells were flow sorted and RNA was purified. Heat map shows differentially expressed genes down- (left heatmap) or upregulated (right heatmap) in injured CAs vs uninjured control vessels.

- **<u>HYPOTHESIS ONE</u>: *Based on these findings, we hypothesize that disease-induced upregulation of Brg1 and HDAC9 drives AdvSca1-SM cells toward an inflammatory, fibrotic phenotype and inhibition of Brg1 and HDAC9 would facilitate AdvSca1-SM cells toward a reparative mature SMC phenotype.***

- Using RNA-Seq approaches, compared to mature SMCs and other adventitial progenitor cell populations, genes associated with hedgehog/Wnt signaling were found overrepresented in AdvSca1-SM cells. As the hedgehog-induced transcriptional regulator, Gli1, was among those uniquely expressed by AdvSca1-SM cells, we took advantage of this to generate Gli1 promoter-YFP reporter mice to construct a novel AdvSca1-SM cell fate-mapping system to selectively track these cells in the context of vascular disease **(Figure 2)**.

- **<u>HYPOTHESIS TWO</u>: *As AdvSca1-SM cells are activated early in response to vascular injury[1], we hypothesize that AdvSca1-SM cells adopt a myofibroblast and/or macrophage phenotype in response to injury to promote the pro-inflammatory, pro-fibrotic environment contributing to vascular remodeling. Gli1-YFP AdvSca1-SM reporter mice will be used to test this.***

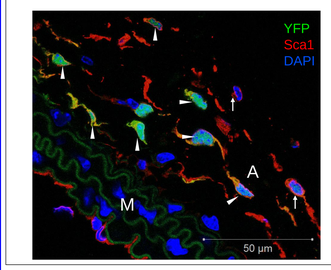


Figure 2. AdvSca1-SM-specific Gli1-Cre^{ERT}-YFP reporter mice. *Gli1-Cre^{ERT}-YFP reporter mice were treated with tamoxifen to induce YFP expression, arteries were harvested and analyzed for YFP (Gli1$^+$), Sca1, and DAPI (nuclei). Following tamoxifen, YFP was expressed exclusively in adventitial Sca1$^+$ cells, but not all adventitial Sca1-positive expressed YFP (i.e. non-SMC-derived AdvSca1 cells were not positive), supporting the use of this system to selectively track AdvSca1-SM cells in response to disease.*

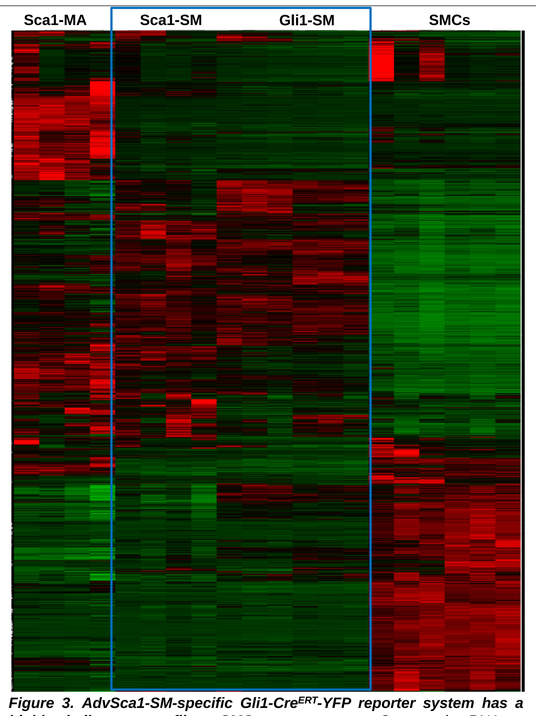


Figure 3. AdvSca1-SM-specific Gli1-Cre^{ERT}-YFP reporter system has a highly similar gene profile as SMC reporter system. *Comparative RNAseq analysis was performed to compare differential gene expression among non-SMC Sca1-MA, SMC Sca1-SM, Gli1-SM, and mature SMC populations. Cluster analysis reveals that the SMC Sca1-SM and Gli1-SM populations exhibit similar gene expression patterns, suggesting they are the same cell population. These data support the use of the Gli1 reporter system to study AdvSca1-SM cells.*

Results

<u>Hypothesis One</u>: Brg1 and HDAC9 are induced in AdvSca1-SM cells following vascular injury.

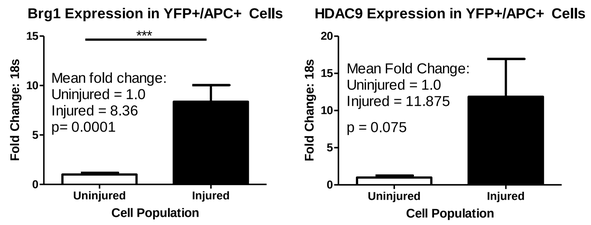


Figure 4. HDAC9 and Brg1 are upregulated in AdvSca1-SM cells 3d after carotid artery ligation injury.
qPCR validation of RNA-Seq data (Figure 1) was performed on SMC reporter mice-derived AdvSca1-SM cells (YFP$^+$/Sca1$^+$). 3 independent experiments were analyzed and each experiment had uninjured and injured arteries pooled from at least 10 mice (M and F). A two-tailed student's t-test was used to test for differences.

Abbreviations

SMC=vascular smooth muscle; Sca1=stem cell antigen; AdvSca1-SM=SMC-derived adventitial Sca1$^+$ adventitial progenitor cell; Tmx=tamoxifen; IF=immunofluorescence; CA=carotid artery; N=arterial neointima; M=arterial media; A=arterial adventitia; ECM=extracellular matrix; CA=carotid artery.

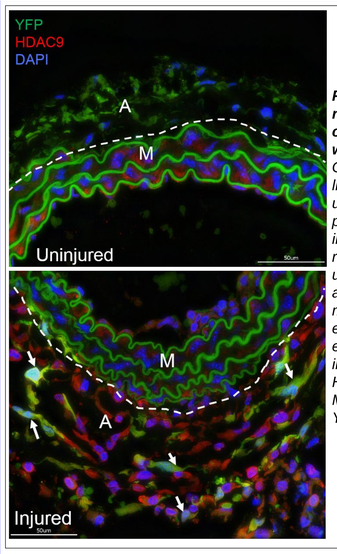


Figure 5. Immunofluorescence staining reveals expansion of AdvSca1-SM cells following injury that is associated with increased HDAC9 expression.
Gli1-SM-reporter mice underwent carotid ligation injury; injured and contralateral uninjured arteries were harvested 3d post-injury and prepared for immunofluorescent staining. These representative images demonstrate uninjured carotid arteries with negligible adventitial staining for HDAC9 and minimal adventitial expansion. Injured CA exhibits robust adventitial remodeling, expansion of AdvSca1-SM cells, and increased adventitial AdvSca1-SM cell HDAC9 expression.
M=media; A=adventitia. Arrows indicate YFP$^+$HDAC9$^+$ AdvSca1-SM cells.

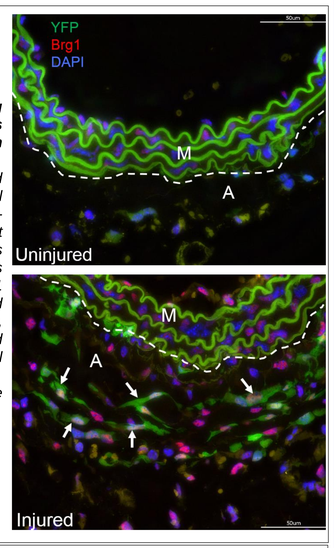


Figure 6. Immunofluorescence staining reveals expansion of AdvSca1-SM cells following injury that is associated with increased nuclear Brg1 expression.
Gli1-reporter mice underwent carotid ligation injury; inured and contralateral uninjured arteries were harvested 3d post-injury and prepared for immunofluorescent staining. These representative images demonstrate uninjured carotid arteries with negligible adventitial staining for Brg1 and minimal adventitial expansion. Injured CA exhibits robust adventitial remodeling, expansion of AdvSca1-SM cells, and increased adventitial AdvSca1-SM cell HDAC9 expression.
M=media; A=adventitia. Arrows indicate YFP$^+$Brg1$^+$ AdvSca1-SM cells.

<u>Hypothesis Two</u>: Gli1$^+$ AdvSca1-SM cells adopt a pathologic phenotype in response to injury.

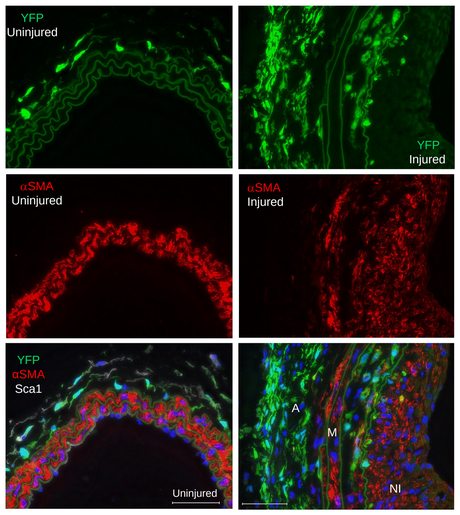


Figure 7. Gli1$^+$ AdvSca1-SM cells greatly expand after ligation injury.
Gli1-Cre^{ERT}-YFP reporter mice were injected with tamoxifen to induce AdvSca1-SM cell-specific YFP knock-in and subjected to CA ligation. Vessels were harvested after 3 weeks. Injured vessels demonstrate a significant expansion of Gli1$^+$ (YFP$^+$) AdvSca1-SM cells that predominantly contribute to adventitial remodeling as well as medial remodeling. These data support the use of this reliable fate-mapping system for tracking AdvSca1-SM cells and illustrate this cell population's propensity to respond in the setting of vascular disease. N=neointima; M=media; A=adventitia. Dashed lines delineate arterial layers.

References

1. Majesky MW, et. al. Differentiated smooth muscle cells generate a subpopulation of resident vascular progenitor cells in the adventitia regulated by Klf4. *Circulation Research* 120:296 (2017)
2. Cardenas CI et al. An HDAC9-MALAT1-BRG1 complex mediates smooth muscle dysfunction in thoracic aortic
3. aneurysm. *Nature Communications* 9:1009 (2018)

The small molecule Brg1 inhibitor PFI-3 attenuates TGF-β induced upregulation of myofibroblast genes in cultured AdvSca1-SM cells

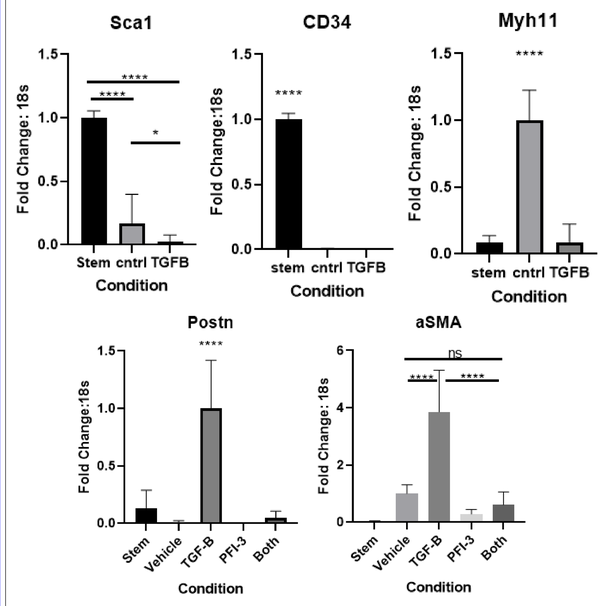


Figure 8. Primary AdvSca1-SM cells express myofibroblast genes when stimulated with TGF-β and the Brg1 inhibitor PFI-3 blocks this effect. *AdvSca1-SM cells spontaneously differentiate into Myh11+ SMCs as measured by decreased Sca1 and CD34 and increased Myh11 in control conditions. TGF-β induces α-SMA and Postn and PFI-3 blunts expression of α-SMA and Postn.*

PFI-3 shows promise at decreasing severity of pathological vascular remodeling *in vivo*

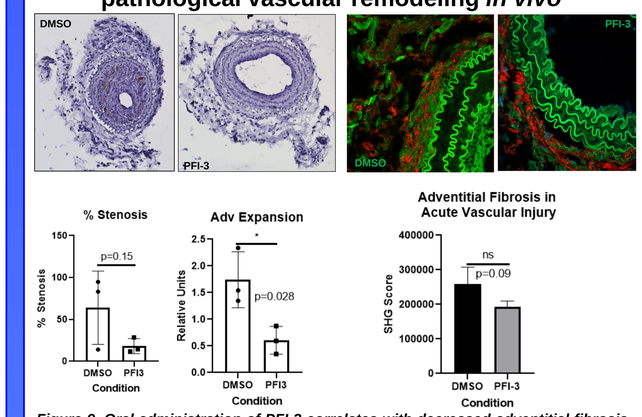
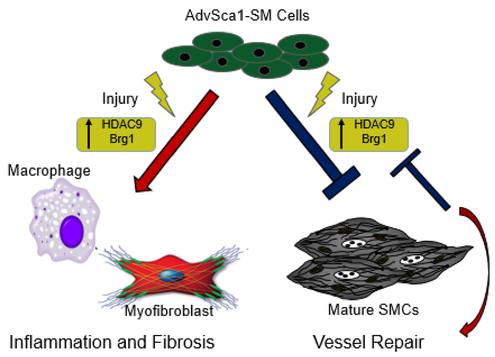


Figure 9. Oral administration of PFI-3 correlates with decreased adventitial fibrosis, adventitial expansion, and neointima formation after carotid ligation challenge. *Gli1-reporter mice underwent carotid ligation injury, one group receiving 50mg/kg of PFI-3 and the control group received 10% DMSO in corn oil via oral gavage.* Vessels were harvested at 4 weeks and quantified for adventitial expansion, percent stenosis, and SHG signal normalized to the diameter of the vessel.

Conclusions



- The epigenetic proteins HDAC9 and Brg1 are upregulated in AdvSca1-SM cells in response to vascular injury – this is supported by RNAseq, qPCR, and IF microscopy.
- Our data support that inhibition of Brg1-HDAC9 blocks AdvSca1-SM cell myofibroblast differentiation and promotes AdvSca1-SM cell differentiation to SMCs

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