The epigenetic remodeling protein Brg1 regulates vascular progenitor cell contribution to chronic vascular fibrosis

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Background

- Cardiovascular diseases cause blood vessels to undergo pathological remodeling and become excessively stiff. This leads to diminished vascular function and decreased quality of life.
- The outer layer of the blood vessel is called the tunica adventitia. We discovered a unique population of stem/progenitor cells that reside in the adventitia. These cells are derived from smooth muscle cells (SMCs) and express the stem marker Sca1 (**AdvSca1-SM cells**).
- In disease states, AdvSca1-SM cells preferentially differentiate into myofibroblasts and contribute to vascular fibrosis.
- Brahma-related gene 1 (**Brg1**) is a chromatin remodeling protein that can insert or eject histones to regulate DNA accessibility for transcription. Our previous data identified Brg1 being upregulated in AdvSca1-SM cells after acute vascular injury.

Hypothesis

Brg1 is activated in response to acute vascular injury and modulates chromatin to preferentially drive AdvSca1-SM cells towards the myofibroblast phenotype. Inhibition of Brg1 will block AdvSca1-SM cell myofibroblast differentiation and decrease pathologic vascular fibrosis.

Materials and Methods

AdvSca1-SM Reporter Mice

- The sonic-hedgehog transcriptional regulator, **Gli1**, is uniquely expressed by AdvSca1-SM cells as compared to other adventitial populations. Taking advantage of this, we developed a lineage-mapping system to permanently label AdvSca1-SM cells with the fluorescent reporter YFP enabling reliable tracking of AdvSca1-SM cells *in situ* (**Figure1**).
- To induce vascular remodeling, complete carotid ligation (**Figure 2**) was performed on the left carotid artery (CA). The carotid ligation is a well-characterized model to generate vascular lesions such as neointima formation, adventitial expansion, and vascular fibrosis. The right carotid artery is left uninjured and serves as an internal control.

Tissue Preparation for Immunofluorescence Microscopy

- Tissues were fixed in 4% paraformaldehyde and sectioned at 6µm. Incubation with primary antibodies was at 4C overnight. Samples were visualized with a Keyence Immunofluorescence microscope.

RNA Extraction and qPCR Experiments

- Total RNA was extracted from purified AdvSca1-SM cells. Sequence-specific primers were designed, and Quantitative real-time PCR was performed with a BioRad CFX96 Real Time System ThermoCycler.

Rigor and Reproducibility

- qPCR experiments were performed on 3 independent biological samples, with each sample containing RNA pooled from at least 10 mice (M and F).
- Power analyses are performed to determine the number of animals needed for *in vivo* studies for statistical significance. Descriptive statistics were calculated by ANOVA or Student t tests.

Results

AdvSca1-SM Cells can be reliably tracked *in situ* using a lineage mapping system driven by Gli1

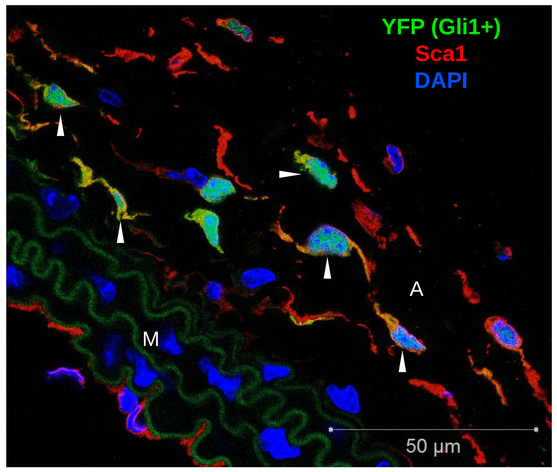
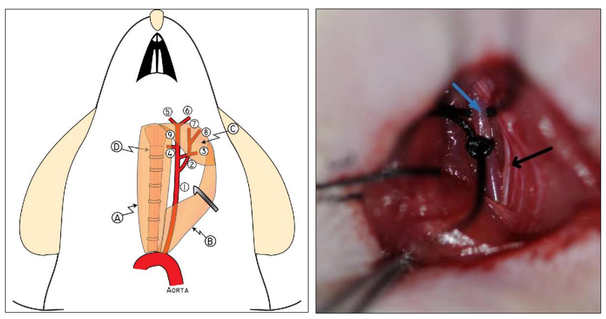


Figure 1. AdvSca1-SM-specific fate-mapping system. *Gli1-Cre^{ERT}-YFP reporter mice were treated with tamoxifen to induce YFP expression, arteries were harvested and analyzed for YFP (Gli1^{+}), Sca1, and DAPI (nuclei). Following tamoxifen, YFP was expressed exclusively in adventitial Sca1^{+} cells, supporting the use of this system to selectively track AdvSca1-SM cells in response to disease. Arrowheads indicate AdvSca1-SM cells which express the YFP reporter and the stem marker Sca1. M= medial layer, A= adventitial layer*

AdvSca1-SM Cells significantly expand in the adventitia and contribute to pathological vascular remodeling after acute carotid ligation.



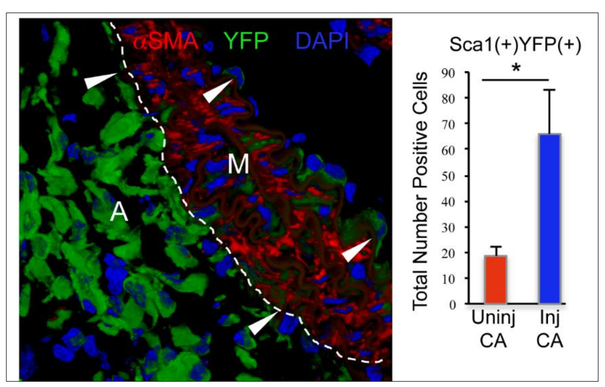


Figure 2. Carotid ligation is a model of acute vascular injury and induces AdvSca1-SM expansion. *Gli1-Cre^{ERT}-YFP reporter mice were treated with tamoxifen to induce YFP expression and subjected to complete ligation of the left CA. Arteries are harvested 4 weeks later for histological analysis. AdvSca1-SM cells expand in the adventitia in response to carotid ligation.*

Brg1 is upregulated at the mRNA and protein level in AdvSca1-SM cells after carotid artery ligation injury

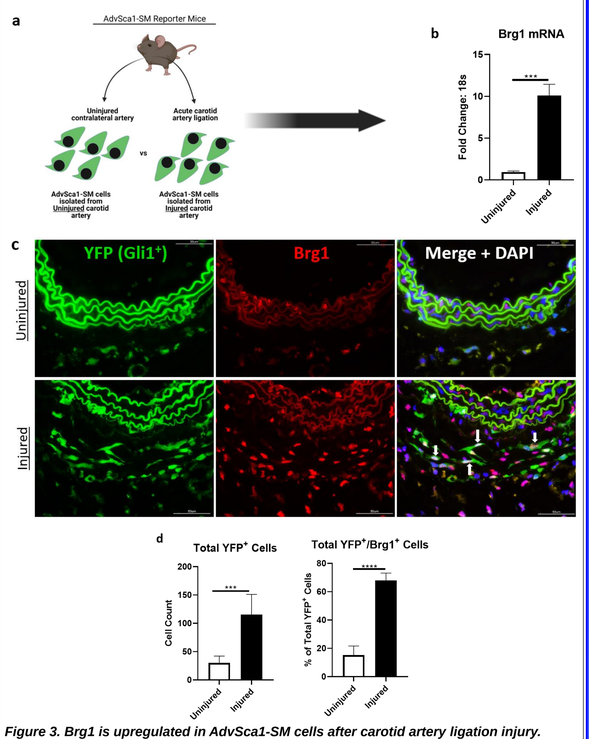


Figure 3. Brg1 is upregulated in AdvSca1-SM cells after carotid artery ligation injury. *AdvSca1-SM cells were isolated from uninjured and injured CAs using flow-sorting and total RNA was extracted for qPCR analysis. In separate studies, tissues were harvested from Gli1-Cre^{ERT}-YFP mice and stained for Brg1. Arrows indicate AdvSca1-SM cells with Brg1 expression in the nucleus. 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.*

Pharmacologic inhibition of Brg1 attenuates injury-induced pathological vascular remodeling

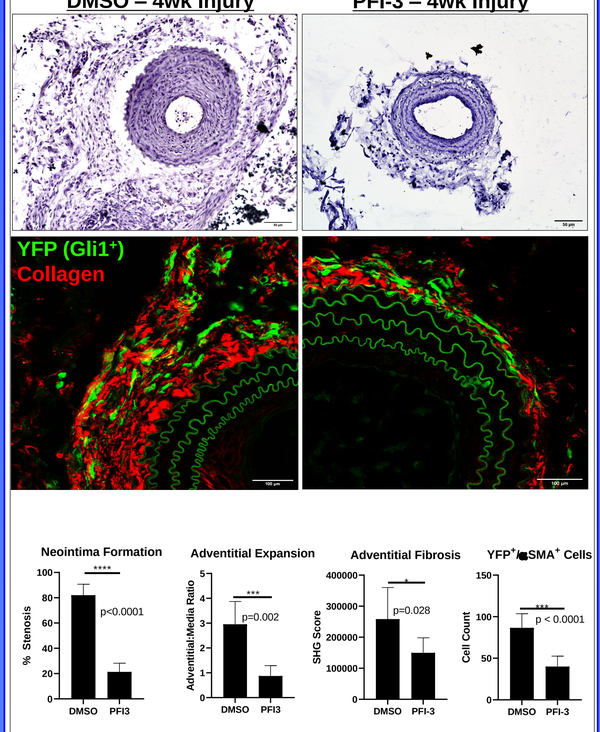


Figure 4. The small molecule Brg1 bromodomain inhibitor PFI-3 decreases adventitial expansion, neointima formation, and vascular fibrosis. Gli1-Cre^{ERT}-YFP mice were subject to carotid ligation injury and separated into 2 groups: control animals received a vehicle solution of 10% DMSO in corn oil, and experimental animals received 50mg/kg PFI-3 via oral gavage. Animals were treated every 4 days and vessels were harvested one month after ligation. Tissues were stained with hematoxylin and label-free second harmonic generation imaging was performed to label perivascular collagen deposition. *3 independent experiments were analyzed, and male and female mice were used equally. A two-tailed student's t-test was used to test for differences.*

Brg1 inhibition blunts TGF-β induced myofibroblast genes in AdvSca1-SM cells

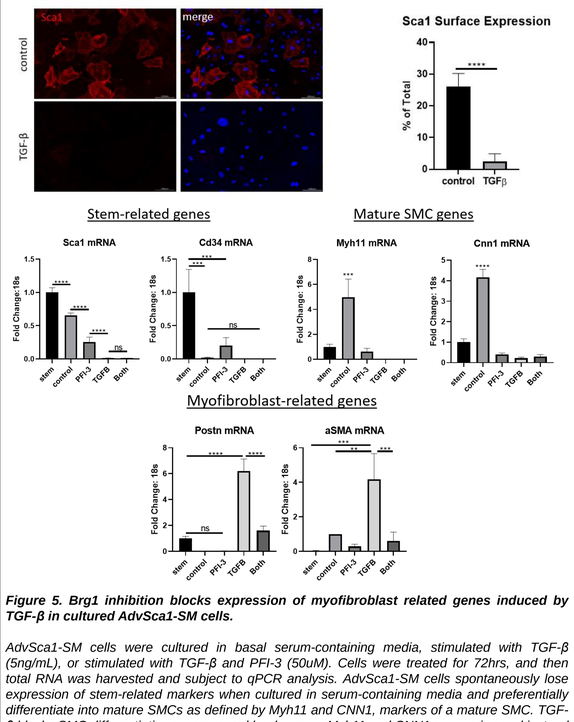
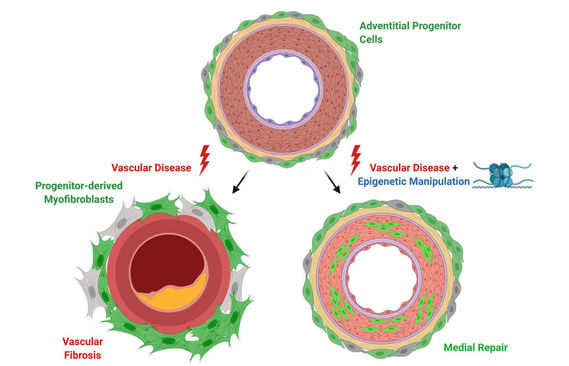


Figure 5. Brg1 inhibition blocks expression of myofibroblast related genes induced by TGF-β in cultured AdvSca1-SM cells.

AdvSca1-SM cells were cultured in basal serum-containing media, stimulated with TGF-β (5ng/mL), or stimulated with TGF-β and PFI-3 (50uM). Cells were treated for 72hrs, and then total RNA was harvested and subject to qPCR analysis. AdvSca1-SM cells spontaneously lose expression of stem-related markers when cultured in serum-containing media and preferentially differentiate into mature SMCs as defined by Myh11 and CNN1, markers of a mature SMC. TGF-β blocks SMC differentiation as measured by decrease Myh11 and CNN1 expression and instead induces expression of myofibroblast genes αSMA and Postn. PFI-3 blocks TGF-β induced myofibroblast differentiation suggesting Brg1 is important for AdvSca1-SM differentiation into myofibroblasts.

Conclusions



- **Brg1 regulates AdvSca1-SM cell contribution to adventitial fibrosis and neointima formation.**
- **Brg1 inhibition decreases adventitial fibrosis, adventitial expansion, and neointima formation after acute vascular injury.**
- **TGF-β drivesAdvSca1-SM → myofibroblast differentiation, and Brg1 inhibition blocks this differentiation.**

Targeting vascular progenitor cell differentiation may confer promising strategies to treat patients who are affected by chronic vascular fibrosis.

- **Future Directions**
  To test Brg1 inhibition *specifically* in AdvSca1-SM cells *in vivo*, Gli1-Cre^{ERT}-YFP mice will be crossed to Brg1$^{flx/flx}$ mice to delete Brg1 only in AdvSca1-SM cells.
  To gain mechanistic insight into the function of Brg1, Cut & Run will be performed to interrogate Brg1-DNA interactions and identify Brg1 binding sites throughout the genome.

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