Role of smooth muscle-derived vascular progenitor cells in atherosclerosis

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Introduction

- Atherosclerosis is a major cause of morbidity and mortality worldwide, but current therapies fail to meet clinical needs.
- Expansion of adventitial microvessels, the vasa vasorum (VV), is believed to drive atherosclerosis progression by facilitating inflammatory cell infiltration.

Image adapted from https://www.earthslab.com/physiology/vasa-vasorum/

- Our group previously identified a unique population of resident stem cells (**AdvSca1-SM cells**) that derive from mature vascular smooth muscle cells (SMCs) and reside in the vessel adventitia.
- AdvSca1-SM cells are selectively enriched for Gli1 compared to other vascular cells. This allowed us to develop the Gli1-Cre/Rosa-YFP mouse model, which selectively and permanently labels AdvSca1-SM cells with YFP, even if they differentiate into other cell types.

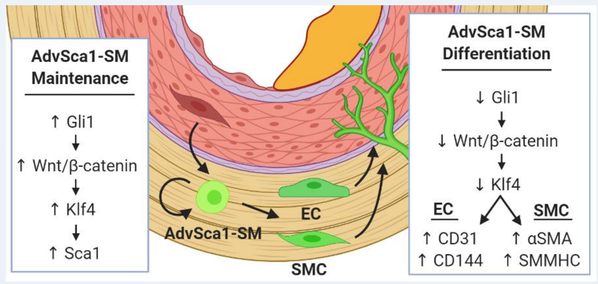
Lu, Sizhao, et al. "Smooth muscle–derived progenitor cell myofibroblast differentiation through KLF4 downregulation promotes arterial remodeling and fibrosis." *JCI insight* 5.23 (2020).

Majesky, Mark W., et al. "Differentiated smooth muscle cells generate a subpopulation of resident vascular progenitor cells in the adventitia regulated by Klf4." *Circulation research* 120.2 (2017): 296-311.

- *In vitro* studies demonstrated that AdvSca1-SM cells can differentiate into SMCs, endothelial cells (ECs), and myofibroblasts.
- *M*atrigel plug assays also showed that AdvSca1-SM cells contribute to in vivo vessel formation via differentiation into SMCs or ECs.

Hypothesis

In atherosclerosis, AdvSca1-SM cells will redifferentiate into smooth muscle or endothelial cells to contribute to vasa vasorum expansion and plaque progression.



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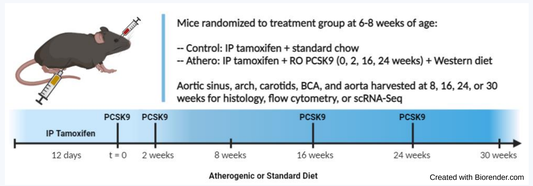
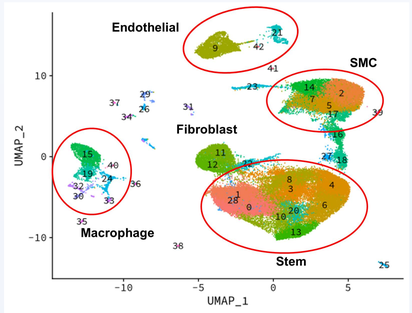
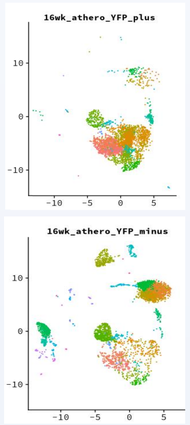
Materials and Methods



All experiments represent a minimum of n=3. Both male and female animals are used for all experiments. Serum is also collected and tested to confirm hyperlipidemia.

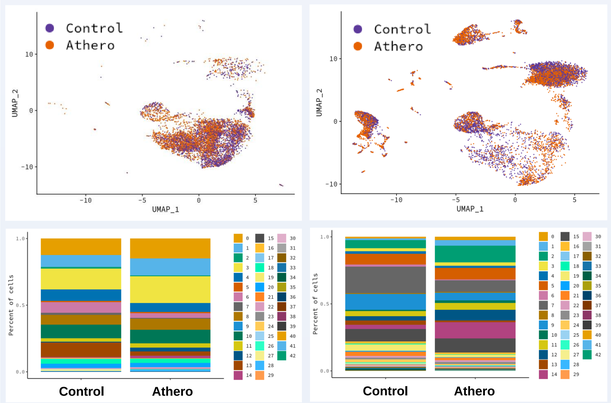
Results





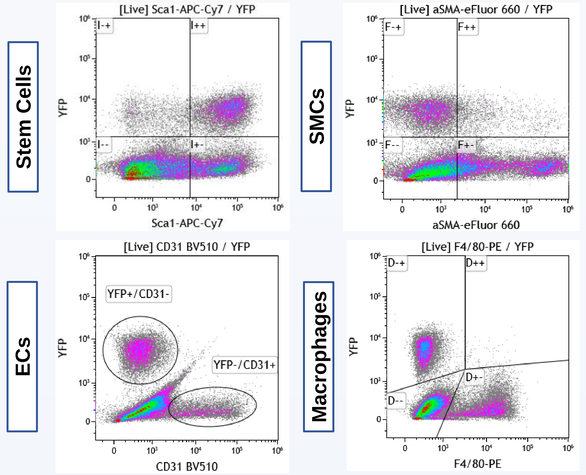
- AdvSca1-SM cells are mainly found in the stem, SMC, and fibroblast clusters.
- There is minimal contribution of AdvSca1-SM cells to ECs or macrophages in atherosclerosis.

YFP Positive **YFP Negative**



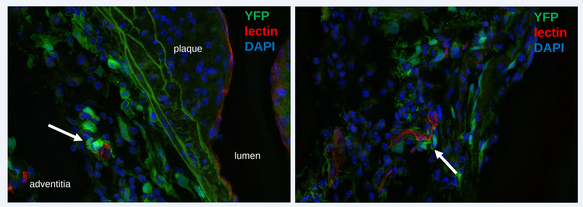
- Although AdvSca1-SM cells (YFP+) primarily remain in the stem clusters, there is a significant shift in the phenotype of those cells in atherosclerosis.
- Additionally, examining the YFP- cells shows the expected changes in SMC and macrophage phenotypes in the setting of atherosclerosis.

Athero, 30 weeks



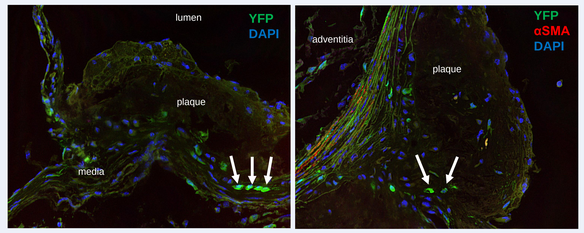
- Confirming our scRNA-Seq data, analysis of the aortic arch, BCAs, and aortic sinus demonstrated that YFP+ cells primarily remain in a stem-like state. However, the AdvSca1-SM cells also differentiate into SMCs.
- Contrary to our initial hypothesis, neither scRNA-Seq and flow data demonstrate appreciable differentiation of YFP+ cells into ECs.
- Finally, AdvSca1-SM cells only rarely gain a macrophage phenotype.

Athero, 16 weeks



- After 16 weeks of treatment YFP+ cells are found associated with functional adventitial microvasculature, as shown with fluorescently labeled lectin injected prior to animal sacrifice.

Athero, 30 weeks



- YFP+ cells were also identified in the media of the vessel, demonstrating that there is a dynamic relationship between SMCs and AdvSca1-SM cells, and suggesting that AdvSca1-SM cells are involved in the maintenance of the media.
- Finally, YFP+ cells were identified in the body of advanced aortic sinus plaques, but the role they play in this context has yet to be determined.

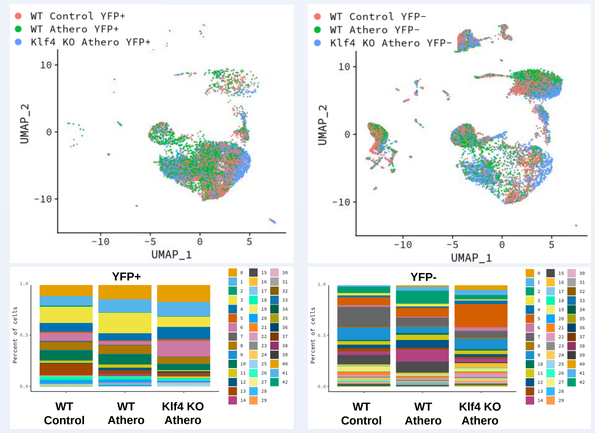
Conclusions

- In both early and late stages of atherosclerosis, there is a large reservoir of AdvSca1-SM cells in a stem-like state. However, this population shows significant phenotypic shifts in atherogenic compared to control conditions.
- When AdvSca1-SM cells differentiate into other cell types in atherosclerosis, they primarily become SMCs or fibroblasts. AdvSca1-SM cells very rarely become either endothelial cells or macrophages.
- AdvSca1-SM cells are associated with functional adventitial microvessels surrounding the aortic sinus after 16 weeks of atherogenic treatment.
- In late stages of atherosclerosis, some YFP+ cells can be found in the core of the plaque. Additionally, AdvSca1-SM cells can be found in the vessel media, demonstrating the dynamic reprogramming between SMCs and AdvSca1-SM cells.

Ongoing Work and Future Directions

- Ongoing work with advanced lesions (24-30 weeks of treatment) will more fully define the functional role and spatial localization of AdvSca1-SM cells in atherosclerotic plaque progression.
- We are also looking at modulating AdvSca1-SM phenotype. Our previous research demonstrated that high levels of the Gli1/Wnt/β-catenin/Klf4 signaling axis converts SMCs into AdvSca1-SM cells and maintains them in that state. We hypothesized that depletion of Klf4 would therefore permit the AdvSca1-SM cells to differentiate into other cell types and contribute to atherosclerosis progression.
- Our preliminary data suggests that Klf4 depletion in AdvSca1-SM cells results in significant phenotypic shifts in YFP+ cells. Some of these shifts resemble WT YFP+ in atherogenic conditions, while others more closely resemble WT YFP+ in control conditions.
- Additionally, Klf4 depletion in AdvSca1-SM cells results in YFP- SMC and macrophage phenotypic shifts, suggesting a potential signaling role and crosstalk for AdvSca1-SM cells with other microenvironmental cells in the setting of atherosclerosis.

YFP Positive **YFP Negative**



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