Contribution of Smooth Muscle-derived Vascular Progenitor Cells to Atherosclerosis

Purpose: Atherosclerosis is a major cause of morbidity and mortality worldwide, but current therapies fail to adequately meet clinical needs. Emerging evidence implicates the outer layer of the blood vessel, the adventitia, in the pathogenesis of atherosclerosis. Specifically, it has been suggested that expansion of adventitial microvessels, the vasa vasorum (VV), drives atherosclerosis by facilitating inflammatory cell infiltration. Our group previously identified a unique population of multipotent resident vascular stem cells (AdvSca1-SM cells) that derive from mature vascular smooth muscle cells (SMCs) and reside in the vessel adventitia, where they are poised to respond to vascular injury. We hypothesized that in the setting of atherosclerosis, AdvSca1-SM cells contribute to VV expansion to drive disease progression.

Methods: We generated a highly specific lineage tracing mouse model to track AdvSca1-SM cells *in vivo* over time. Lineage tracing mice were placed on either normal chow or modified Western diet for 8 to 30 weeks, then vascular tissue was analyzed using IF microscopy, scRNA-Seq, and flow cytometry.

Results: scRNA-Seq and flow cytometry revealed a large reservoir of AdvSca1-SM cells in a stem-like state in the setting of atherosclerosis. However, this population shows significant phenotypic shifts in atherogenic conditions and influences the transcriptional profiles of non-AdvSca1-SM-derived cells. When AdvSca1-SM cells differentiate into other cell types, they primarily become mature SMCs, modulated SMCs, and myofibroblasts. Contrary to our preliminary data, AdvSca1-SM cells very rarely differentiate into endothelial cells.

Conclusions: AdvSca1-SM cells in atherosclerosis predominantly differentiate into SMCs and myofibroblasts, or else remain in a stem-like state. Future studies will further define the functional role of AdvSca1-SM cells in plaque progression.