Role of smooth muscle-derived vascular progenitor cells in 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 progression 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 in order to track AdvSca1-SM cells *in vivo* even if they differentiate into other cell types. Lineage tracing mice were placed on either normal chow or Western diet for 8 or 16 weeks, then vascular tissue was analyzed using IF microscopy, scRNA-Seq, and flow cytometry. Results: scRNA-Seq and flow cytometry revealed that AdvSca1-SM cells in atherosclerosis primarily differentiate into mature SMCs, modulated SMCs, and myofibroblasts. Contrary to our preliminary data, AdvSca1-SM cells very rarely differentiate into endothelial cells. Additionally, despite previous evidence of SMCs gaining a macrophage-like phenotype in atherosclerosis, we identified only rare instances of AdvSca1-SM cells contributing to macrophage populations. Conclusions: As in our findings in acute vascular injury, AdvSca1-SM cells in atherosclerosis predominantly differentiate into SMCs and myofibroblasts. Future studies on advanced lesions will define the functional role of AdvSca1-SM cells in atherosclerotic plaque progression.