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Origins of smooth muscle progenitor cells in transplant arteriosclerosis

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Introduction

The arterial adventitia harbors a niche environment that maintains resident vascular progenitor cells. Originally identified in the aortic adventitia of *Apoe*$^{-/-}$ mice,[1] it is now clear that adventitial progenitor cells (AdvPCs) are a heterogeneous mixture with multiple fate potentials. In fact, the *combined* fate potentials of all AdvPC subtypes described to date is sufficient to produce essentially all resident cell types of the artery wall. Therefore, the capacity of the adventitia to repair the wall and contribute to vascular disease is much greater than commonly thought.

Adventitial Progenitor Cell Subsets

The first AdvPC subset to be identified, referred to now as AdvSca1^{sm}, is fate-restricted to differentiate to smooth muscle cells (SMCs) and pericytes.[1–3] A second subset, referred to as AdvSca1^{ma}, is CD45-positive and can differentiate to tissue resident macrophages in the artery wall.[4] This latter subset also has vasculogenic potential and contributes to formation of adventitial and periadventital microvessels *in vivo*.[4] In addition, adventitial progenitor cells expressing CD34[1,5], VEGFR2[5], PDGFRβ[2], Gli1-cre[6], KLF4[2,3], and CD146[7] have been reported. However, among the first markers identified for AdvPCs is the cell surface receptor tyrosine kinase *c-kit*.[1]

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Disclosures

None

In the present study, Ni et al[8] report that *c-kit*$^{+}$ cells could be found in both the CD45^{+} and CD45^{-} fractions of AdvPCs. This suggests that *c-kit*$^{+}$ AdvPCs possess multiple fate potentials *in vivo*. To directly test this possibility, Ni et al[8] used Kit-CreER;Rosa26-tdTomato mice pulsed with tamoxifen and examined one week after the last tamoxifen injection. Most of the tdTomato^{+} cells were found in the adventitia where ~70% were Sca1^{+} and ~40% were CD45^{+}. These results suggest that both AdvSca1^{sm} and AdvSca1^{ma} express *c-kit in vivo,* potentially forming a third subset of AdvPCs.

Origins of Neointimal SMCs: Role of Adventitial *c-kit*-positive Cells

The precise origins of intimal SMCs are debated. There are several reasons for this: (1) Human disease-prone arteries have preexisting intimal thickenings from which atherosclerotic plaque SMCs are thought to arise. Murine arteries rarely have preexisting intimal SMC masses, a reminder that the mouse does not fully model human atherogenesis. (2) Neointimal SMC origins may be injury model-dependent based on the severity of medial damage.[1,9,10] (3) Movement of adventitial cells to the intima may be more likely in thin walled murine arteries than in thick-walled human arteries. (4) In human plaques and several mouse models studied to date, intimal SMCs arise by mono- or oligo-clonal expansion via mechanisms that are not understood.[11–13] Therefore, the fate of *c-kit*-positive AdvPCs in injured murine arteries is of particular interest. To address this question, Ni et al[8] used Kit-CreER;Rosa26-tdTomato mice and an aortic allograft mouse model of transplant arteriosclerosis. Organ transplantation is often the last hope for patients suffering from end stage organ failure. Unfortunately, long-term survival of transplant recipients is limited by an accelerated form of vasculopathy referred to as transplant arteriosclerosis. Given the extensive cell death that occurs in transplanted artery walls, the origins of intimal SMCs in these transplants is not immediately obvious.

To test whether *c-kit*$^{+}$ cells contribute to neointimal SMCs in transplanted arteries, the authors employed interposition grafts of aortic segments from Balb/c mice into carotid arteries of recipient C57Bl/6J mice.[8] This allograft transplant model produces severe vascular injury caused by immune cell responses leading to extensive cell death in all layers of the grafted arterial segment.[8] When harvested one month after transplantation, the donor segment from wild-type Balb/c mice was heavily invested with tdTomato-positive *c-kit* lineage cells of recipient origin. Neointimal SMCs in the central graft segment contained 10.6+/−1.2% tdTomato^{+}/SM22^{+} SMCs. By contrast, only 0.1% of SMCs in carotid media distant from the site of the aortic allograft were tdTomato^{+}. As shown previously, the origin of SMCs in the grafted segment is from adjacent segments of recipient artery wall and not from circulating SMC progenitors. Consistent with such an origin, tdTomato^{+} cells were most abundant (30.9+/−4.4%) in the grafted segment immediately adjacent to the recipient artery wall and bearing the suture site.[8] Given the low frequency of Kit-CreER;Rosa26-tdTomato^{+} cells in the carotid media prior to grafting, compared with their relative abundance in the adventitia of the recipient artery, the authors logically suggest that neointimal tdTomato^{+} SMCs most likely originated from the recipient adventitia. This conclusion was supported by results from the reverse experiment where a donor segment from Kit-CreER;Rosa26-tdTomato^{+} mice was transplanted to recipient carotid arteries of unlabeled Balb/c mice. In this case, strikingly, the neointimal SMCs that appeared in the

aortic allograft segment contained no detectable Kit-CreER;Rosa26-tdTomato$^+$ cells. Additional experiments involving reciprocal bone marrow chimeras showed that marrow-derived *c-kit*$^+$ cells produced CD45$^+$ leukocytes and not SMCs.

Cells Expressing AdvPC markers are Present in Normal Media

Of interest was the observation that ~0.1% of normal medial cells were Kit-CreER;Rosa26-tdTomato$^+$. As Ni et al[8] point out, a contribution of rare pre-existing medial *c-kit*$^+$ cells to the transplant neointima cannot be ruled out. Scattered, rare Sca1$^+$ cells were previously reported in the media of healthy vessels by Sainz et al.[14] More recently, Dobnikar et al[15] employed a 10X single cell sequencing platform to show that normal mouse aortic media contained 0.2%−1.6% Sca1$^+$ cells. Further analysis showed that medial Sca1 expression marked SMCs switching from a contractile phenotype to a more synthetic phenotype *in vivo*. The rare occurrence of these cells in the media combined with their non-contractile phenotype may have important implications for SMC clonal expansion in murine arteries.[12,13,16]

Stem Cell Factor/cKit Signaling Promotes AdvPC Migration *in vitro* and Neointimal Thickening *in vivo*

Ni et al[8] next performed studies to address whether or not *c-kit* plays a functional role in neointimal development. *c-kit* is a receptor tyrosine kinase whose kinase activity is responsive to the ligand stem cell factor (SCF). In cell culture studies Ni et al[8] showed that SCF promotes *c-kit*$^+$ AdvPC migration, but not proliferation or differentiation. Thus, SCF acts as a chemotactic factor for *c-kit*$^+$ AdvPCs *in vitro.* To assess the role of *c-kit* activity in neointima formation, the authors used a *c-kit* function-blocking monoclonal antibody (ACK2) suspended in slow-release pluronic gel and applied to the adventitial side of the grafted segment. Four weeks after transplant neointimal area, relative cell number, and percent of neointimal SMC that are tdTomato$^+$ were all reduced by treatment with monoclonal antibody ACK2.[8]

SMC Differentiation from Adventitial cKit-positive Cells Requires Metabolic Reprogramming

In addition to SCF, TGFβ was also increased in blood plasma and neointima of allograft transplanted mice.[8] TGFβ stimulated differentiation of *c-kit*$^+$ AdvPCs to SMCs. Differentiation was accompanied by increased glucose uptake and rapid acidification of the extracellular media by increased lactate production suggesting a shift to glycolytic metabolism. Next, the authors used 2-deoxy-glucose (2DG), a glucose analog that cannot be further metabolized, to block glycolysis. 2DG inhibited increases in *Acta2*, *Cnn1*, and *Tagln* expression indicative of a block to SMC differentiation in these cells. Moreover, siRNA-mediated knockdown of the rate-limiting glycolytic enzyme hexokinase-1 (HK1), but not HK2, also blocked *c-kit*$^+$ cell differentiation to SMCs.

A link between products of cellular metabolism and epigenetic pathways critical for cell fate specification has been reported for endothelial cells[17] but not for SMCs. Ni et al[8]

specifically tested a role for hexosamine-derived UDP-GlcNAc, a substrate for protein-O-GlcNAcylation, in SMC differentiation from *c-kit*$^+$ AdvPCs *in vitro.* The authors found that both myocardin and SRF were O-GlcNAc modified in response to TGFβ stimulation, and that SMC differentiation was blocked by 6-diazo-5-oxo-L-norleucine (DON), an inhibitor of protein-O-GlnNAcylation. They conclude that metabolic reprogramming of adventitial progenitor cells is required for SMC differentiation *in vitro.*

Summary and Questions Going Forward

Much additional work is needed to identify the metabolic pathways that control SMC differentiation. O-GlcNAc protein modification is reversible and the on/off kinetics of O-GlcNAcylation of SMC transcription factors will be a point of regulation needing further definition. In addition, the precise mechanisms by which metabolites modify SRF, myocardin, or various epigenetic targets involved in SMC differentiation need to be further characterized. If AdvPCs actually do function to repair artery walls, they would need to migrate within those walls to do so. Evidence is growing to suggest that the outer media and inner adventitia exhibit properties of a functional unit with demonstrable cell movements between compartments.[3,9] The present study adds to a number of previous reports[1,6,7] suggesting that AdvPCs in the mouse can move through the media and into the neointima, at least in severely injured or transplanted vessels. At the same time, recent reports of SMC clonal expansion in neointima formation leave open a possible role for rare medial cells that express AdvPC markers *in vivo*.[12,13,16] Future work will determine relative contributions of SMC progenitor cells resident in the media and the adventitia to maintenance, repair, and disease of the artery wall.

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