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Transcriptomic profiling of the mouse aorta to identify novel cellular drivers of aortic stiffening in hypertension

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Aortic stiffening is a hallmark of hypertension that manifests from changes to both functional (vasoconstriction) and structural (vascular fibrosis and hypertrophy) properties. Several key cell types are known to play a role in modulating these changes, however the cellular mechanisms are poorly understood. Therefore, this study aimed to characterize the cellular landscape of the hypertensive mouse aorta and identify the cellular subsets that drive functional and structural changes in aortic stiffening. Twelve-week-old male C57BL/6 mice were randomly assigned to receive angiotensin (Ang) II- (0.7 mg/kg/day) or vehicle (saline)-infusion via osmotic minipump (*s.c.*). After 28 days, mice were killed, and aortae harvested and prepared for single-cell RNA sequencing using Chromium 10x and NovaSeq genomics platforms. We identified 17 cell types in the aorta, some of which were further subclustered based on their unique gene profile, resulting in 41 distinct subclusters. Of the major aortic cell types including endothelial cells (2 subclusters), vascular smooth muscle cells (2 subclusters) and immune cells (15 subclusters), fibroblasts were undoubtedly the most abundant (14 subclusters). Importantly, a novel fibroblast subcluster that uniquely expressed the profibrotic gene *Cthrc1* was 60-fold more abundant in aortas from Ang II- *cf.* vehicle-treated mice. Compared to all fibroblasts in the hypertensive mouse aorta, collagen genes (*Col1a1*, *Col1a2*, *Col3a1*) were most highly expressed by *Cthrc1*$^+$ fibroblasts. Gene ontology revealed upregulation of profibrotic signalling pathways in *Cthrc1*$^+$ fibroblasts (i.e. extracellular matrix and collagen fibril organization and cell migration) when compared with all fibroblast subclusters. Immunohistochemistry localized CTHRC1 protein in the adventitia of hypertensive mouse aortae but not in that of vehicle-control mice. Of the immune cell types, macrophages, B cells and T cells were the most abundant subtypes. Interestingly, of the 4 distinct "macrophage-like" subpopulations in the aorta one displayed a gene expression profile reminiscent fibrocytes (*Cd34*, *Pdgfra*) with a strong fibrogenic phenotype highly expressing collagen. Flow cytometry analyses revealed that M2-like macrophages positive for collagen-1 (CD45$^+$F4/80$^+$CD206$^+$Col1$^+$; likely fibrocytes) are indeed upregulated in hypertension. We report the first ever comprehensive analysis of the whole aortic cellulome in the setting of hypertension. The identification of a novel profibrotic fibroblast that is uniquely expressed in the hypertensive mouse aorta and upregulation of profibrotic fibrocytes in hypertension raises the exciting possibility that these cell types may be key drivers (and potential therapeutic targets) of aortic stiffening.