Cellular senescence impairs the innervation of the aging heart

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Aging is a major risk factor for impaired cardiovascular health. The aging myocardium is characterized by endothelial cell dysfunction, increased hypertrophy and fibrosis and electrophysiological alterations that predispose the elderly to arrhythmic risk. The mechanism of age-associated pathophysiological alterations are incompletely understood. Previous studies showed impaired endothelial cell functions associated with senescence and alterations of the vasculature in aging. Since vessels align with nerves and this interplay is critical for tissue homeostasis in other organs, we investigated whether an impairment of the neuro-vascular interface in the aging heart may contribute to age-associated pathologies.

To investigate the innervation of the aging mouse heart, we assessed the cardiac autonomic nervous system histologically in 12-weeks and 20-months old mice using the pan-neuronal marker Tuj1 and the sympathetic marker tyrosine hydroxylase. Interestingly, sympathetic innervation of the left ventricle, especially around arteries, was reduced by 0.66±0.07-fold with aging (n=9; $p<0.05$). CGRP-positive sensory fibers were also reduced by 0.41±0.09-fold in the left ventricle indicating that aging reduces innervation in the left ventricle (n=3; $p<0.01$). Similar findings were observed in G4 telomerase-deficient mice, which develop a pre-mature senescent phenotype (reduced innervation by 0.48±0.09-fold, $p<0.01$, n=4). Electrophysiological studies confirmed an increased incidence of ventricular arrhythmias in old versus young hearts.

To determine a potential contribution of neurovascular cellular cross-talk, we analyzed gene expression of endothelial cells in the aging mouse heart by bulk RNA sequencing. Aging significantly induced genes associated with GO-terms related to neuronal cell death and axon injury. Sema3a, a known axon repelling

factor, was significantly increased by 1.5±0.2-fold in endothelial cells of aged mice hearts, a finding which was confirmed by RT-qPCR of isolated endothelial cells and by histology ($p<0.05$).

Interestingly, *Sema3a* expression is also induced in senescent endothelial cells in vitro, suggesting a putative role for senescence-induced alterations of gene expression in impaired cardiac innervation. Indeed, we observed an increase in acidic β-galactosidase activity as a marker for cellular senescence in the aging heart, in particular around arteries. To eliminate senescent cells and to see, if the left ventricular axon density can be restored, we treated 18-month old mice with a combination of the two senolytic drugs dasatinib and quercetin (n=5), which are known to deplete senescent cells in vivo. Indeed, after 2 month of treatment, we identified a reduction in cellular senescence in the aging heart by 0.4±0.1-fold ($p<0.05$) and a restoration of ventricular innervation compared to placebo treated mice by 1.8±0.4-fold. In line with these findings, endothelial cell function, as assessed by ex vivo aortic ring assays, was restored in aged mice receiving the senolytic cocktail by 3.5±0.7-fold. Finally, endothelial *Sema3a* expression and other repulsive genes were reduced by senolytic treatment.

In conclusion, we demonstrate that aging augments axon repelling signals in endothelial cells and reduces axon density in the left ventricle. The depletion of senescence cells prevented age-induced impairment of innervation suggesting a critical role of senescence-associated factors in axon repulsion in the aging heart.