Title: Smooth Muscle Cell Sirtuin 6 Knockdown Causes an Inflammatory Adventitial Response of the Ascending Aorta in a Mouse Model of Large Vessel Vasculitis

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Structured Abstract:

Background: Cardiovascular disease is a growing global epidemic as one of the top six causes of preventable deaths identified by the World Health Organization. It is now known that cardiovascular disease is strongly associated with inflammation. A vital illustration of this are the primary inflammatory diseases of the vascular system, characterized by vasculitis. These conditions carry a high disease burden and have devastating consequences including aortic rupture, tissue infarction, organ failure, and blindness. However, the underlying basis for vasculitis remains unknown. Recently, the sirtuin family of nicotinamide adenine dinucleotide (NAD)-dependent enzymes has been linked to both vascular disease and inflammatory cascades. Among the sirtuins, sirtuin 6 (SIRT6) is uniquely required for mouse survival and its absence leads to organ inflammation. Whether SIRT6 participates in vascular inflammatory signaling is unknown.

Methods: To study the role of SIRT6 in vascular smooth muscle cells (VSMC) within the medial lamina of elastic vessels we created a novel strain of inducible VSMC-specific SIRT6 deficient mice using Cre-loxP technology. At 6 months of age the animals were sacrificed and tissues were harvested under pressure fixation using 4% paraformaldehyde, followed by paraffin embedding and microtome sectioning. Finally, these tissue sections were treated with H&E, Movat’s stain, and immunostaining. Subsequently, slides were analyzed using light and fluorescent microscopy. SIRT6 knockdown in primary cell cultures of aortic smooth muscle cells was achieved by administration of adenovirus mediated Cre delivery. Knockdown efficiency and associated gene expression changes were measured via real time quantitative PCR (RT-qPCR).

Results: Inflammation of the affected blood vessels were characterized by a pronounced increase in CD45+ cells in the adventitial layer – a marker of hematopoietic immune cells. Further characterization revealed an increase in a sub-population of CD3+ cells, a marker of lymphocytes, and CD68+ cells, indicative of macrophages. Qualitatively, this inflammatory invasion of the adventitial layer of blood vessels was associated with the degenerative changes such as elastin degradation and collagen deposition, as well as changes in vessel wall thickness and area. Primary cell cultured based experiments reveal efficient SIRT6 knockdown with an associated increase in inflammatory cytokines, such as IL-1β.

Conclusions: Our findings have identified a striking inflammatory destruction of the vessel walls in SIRT6 deficient mice, particularly involving the aorta. The inflammatory nature of SIRT6 deficient SMCs was confirmed via in vitro experiments. This unique preliminary data suggests that SIRT6 within vascular SMCs may control vascular inflammation.