Investigating the Role of STING in Response to Cardiovascular Inflammation

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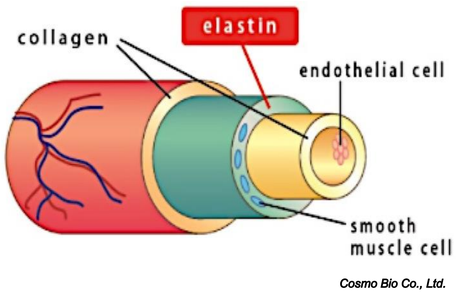
Abstract

Advances in our understanding of innate immune signaling demonstrate that the Stimulator of Interferon Genes (STING) senses cellular damage upon injury or infection and, through the activation of Type I interferon genes, invokes a subsequent pro-inflammatory response[1]. Although STING has been studied as a gain-of-function mutation in the context of STING-associated vasculopathy[2], its role in endothelial cells in response to vascular injury remains largely unexplored. Studying endothelial STING through this lens will help better understand its role in the effects of angioplasty and stent implantation, commonly-used procedures to improve blood flow in Peripheral Artery Disease (PAD). In mouse models of angioplasty, to compensate for the denudation of the vascular endothelium, elastin, an extracellular matrix protein indicative, in large quantities, of vessel stiffening and adverse remodeling[3], can be quantified; elastin has also been used in relevant literature as a metric of wire-induced vessel remodeling[4].

I hypothesize that knocking out the STING gene has a protective effect against excess elastin deposition in arteries following wire-mediated denudation of the endothelium in a mouse model of angioplasty.

In vivo experiments using a model of angioplasty in the carotid arteries of both WT and STING -/- mice demonstrate that without an injury stimulus, STING does not contribute to elastin deposition in vessels. The elastin readouts for the control groups are in line with literature on the wire procedure[4]. Finally, this preliminary study showed that vessel medial area in STING-/- injured arteries are not different from STING-/- uninjured arteries, though there were not sufficient mice to perform statistical analysis. Nevertheless, this similarity in elastin deposition could suggest that vascular STING may contribute to sensing wire-mediated injury.

Analyzing recently added data to this ongoing experiment will be used to compare between STING-/- and WT injured arteries, and determine whether STING signaling is involved in injury sensing. STING signaling pathways can then be modulated to mitigate angioplasty-invoked vessel damage.



Elastin in a Blood Vessel

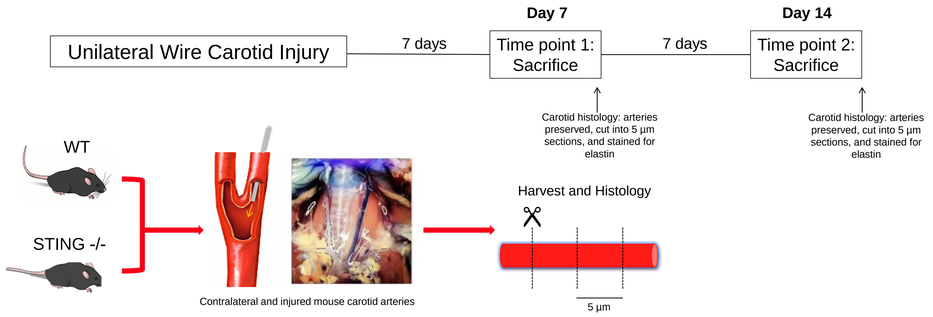
Elastin provides elasticity to large blood vessels like the carotid arteries to protect them from pressure changes, but large increases in elastin mean stiffer vessels; they imply large quantities of smooth muscle cells, primary elastin producers *and* large manufacturers of vessel-stiffening proteins like collagen.

Objectives

- Achieve fluency in ImageJ image-analysis techniques.
- Gain literacy in project-related topics— including PAD and stent implantation, elastin and collagen deposition, immune cell interactions with and transmigration through the endothelium, and STING activation and cellular effects— from primary scientific sources.
- Develop as deep an understanding as possible of the laboratory procedures resulting in the image analyzed in ImageJ.
- Through this background research, generate a sound hypothesis for the effect of STING in the damaged endothelium, and with subsequent image analysis, draw preliminary conclusions, troubleshoot, and propose next steps.

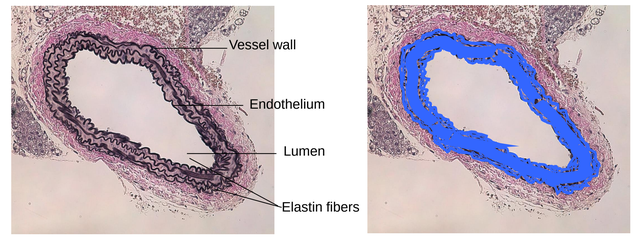
Methods

Experimental Design



STING -/- and WT mice were subjected to unilateral wire carotid injury and sacrificed 7 or 14 days post-injury. Mice's injured and contralateral uninjured arteries were extracted, cut into 5 µm sections, and stained for elastin with Verhoffs Van Gieson. Stained arteries were imaged, and images were analyzed using ImageJ.

Quantifying elastin by vessel medial area in ImageJ



ImageJ software was used to calculate vessel medial area to quantify dark purple elastin residing between the endothelium and vessel wall. The area encompassed by innermost layer of elastin was measured using the "freehand selection" tool and subtracted from that by the outermost to calculate vessel medial area. Vessel medial area is represented by the area shaded in blue in the right image.

Results

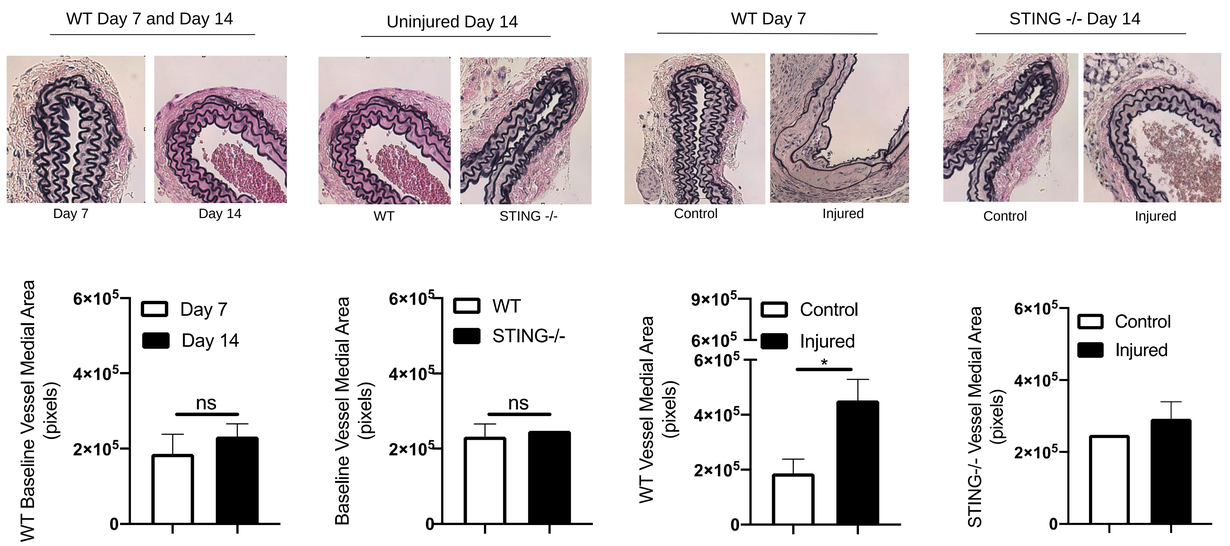


Figure 1. Vessel remodeling responses to wire injury unchanged in STING-/- mice

Injured arteries were isolated from WT and STING -/- mice 7 and 14 days, respectively, after subjected to unilateral carotid injury. Uninjured control arteries were isolated from both groups at both timepoints. Histological, transversal sections of 5-µm thickness were obtained and stained to visualize elastin fibers, and vessel medial area was quantified. Representative elastin-stained carotid artery sections are shown above at 40X magnification and cropped by 60-80% to remove fat and muscle areas. Bars represent an average medial area for all animals with SEM. *P<0.001 versus uninjured.

N=3 for WT control Day 7; N=3 for WT control Day 14; N=1 for STING-/- control Day 14; N=3 for WT injured Day 7; N=3 for STING-/- injured Day 14.

Conclusions

Our preliminary data exhibit a trend indicating the inability of STING-/- mice to sense wire injury, as evidenced by absence of vessel medial area in response to injury.

More mice are required to compare vessel medial area in STING-/- injured arteries with that of WT injured arteries to demonstrate that STING signaling is involved in injury sensing in blood vessels.
Better understanding the role of STING signaling in blood vessel remodeling post-angioplasty will help doctors treat patients with PAD without long term side effects.

Future Work

- Vessel medial area will be quantified in additional mice.
- Future experiments using live cell microscopy studies will be used to translate these *in vivo* observations into mechanistic findings.

References

[1] Ahn J & Barber G. 2019. STING signaling and host defense against microbial infection. *Experimental & Molecular Medicine.* 51:155. DOI: 10.1038/s12276-019-0333-0
[2] Liu Y, Jesus AA, Marrero B, Yang D, Ramsey SE, Montealegre Sanchez GA, Tenbrock K, Wittkowski H, Jones OY, Kuehn HS et al. 2014. Activated STING in a Vascular and Pulmonary Syndrome. *New England Journal of Medicine*. 371(6): 507–518. DOI: 10.1056/NEJMoa1312625
[3] Robert L, Jacob MP, Fülöp T. 1995. Elastin in blood vessels. *Ciba Found Symp*. 192:286-303. DOI:10.1002/9780470514771.ch15
4. Pruthi D, Khankin EV, Blanton RM, Aronovitz M, Burke SD, McCurley A, Karumanchi SA, Jaffe I. 2015. Exposure to Experimental Preeclampsia in Mice Enhances the Vascular Response to Future Injury. *Hypertension*. 65(4): 863–870.

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