

Second Harmonic Generation and Multiphoton Excited Fluorescence Microscopy as a Phenotypic Tool in an Animal Model.

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We have developed a novel animal model in which injury induced neointimal hyperplasia and vascular reactivity were isolated within a Quantitative Trait Loci (QTL) on the q-arm of rat chromosomes 3 (RNO3) in an (SHR X BN) population through linkage analysis [1]. We validated the RNO3 QTL by substitution mapping with a SHR.BN3 congenic strain, which demonstrated significant differences in injury induced neointimal hyperplasia and vascular reactivity compared to the parental SHR [2]. The development of neointimal hyperplasia is a result of the inflammatory cascade that occurs upon injury and/or insult resulting in a fibroproliferative response within the vascular wall. The injury response includes the migration and proliferation of smooth muscle cells and the accumulation of extracellular matrix leading to vascular occlusion. Also isolated within the RNO3 QTL in the SHR and SHR.BN3 strain was a significant difference in the vasoconstrictive response of endothelium denuded carotid rings. Taken together, we have isolated the genetic element responsible for the development of neointimal hyperplasia and vascular reactivity on RNO3 associated with these two strains.

Collagen and elastin make up the arterial wall and are oriented differently in the intima, media and adventitia of the artery. The media layer consists of concentric elastic fibers and collagen that is bordered by the internal and external elastic lamina. The adventitia contains large collagen bundles and provides a load bearing structural scaffold for the artery. It has been previously shown that collagen fiber orientation in the adventitia has a significant effect on the arterial mechanical response [3]. We hypothesize that the vascular differences in the development of neointimal hyperplasia and vascular reactivity in the SHR and SHR.BN3 strains are associated with the viscoelastic organization and properties of the arterial wall. Consequently, to evaluate these properties, we assessed the plausibility of using second harmonic generation (SHG) and multiphoton excitation (MPE) fluorescence microscopy as a quantitative imaging tool, to assess the vascular organization in these strains.

Iliac arteries of the SHR and SHR.BN3 strains were perfused with PBS and fixed in 10% neutral buffered formalin. Individual arteries were pre-embedded in 3% agarose followed by paraffin processing, embedding and sectioning. Sections were de-paraffinized and stained with DRAQ5 (BioStatus) and coverslipped with Eukitt (Electron Microscopy Sciences). Arterial cross sections were imaged using a Leica TCS SP5 laser scanning confocal microscope (Leica Microsystems, Bannockburn, IL) equipped with a Ti:sapphire tunable multiphoton laser (Coherent, Santa Clara, CA). Images were acquired in a sequential manner with an excitation wavelength of 860nm to generate second-harmonic generation (SHG) from collagen at 430 nm (425-435 nm). An excitation wavelength of 820nm was used for multiphoton-excited fluorescence (MPE) of elastin at 500nm (495-525 nm). Nuclei were excited with a conventional 633 laser and collected at a 640-655nm (Figure 1).

Using multiphoton confocal microscopy, we successfully compared the SHR and SHR.BN3 strains and found significant differences in adventitial collagen content and organization using SHG microscopy (Figure 1). Analysis software packages for assessing collagen content and bundling will be discussed as

used in this model to confirm the differences in extracellular matrix remodeling. These findings confirm that SHG microscopy can be successfully employed in animal models of disease to qualitatively and quantitatively assess extracellular matrix differences. As a result, a direct link between injury-induced neointimal hyperplasia, vasoreactivity and ECM remodeling was confirmed.

References:

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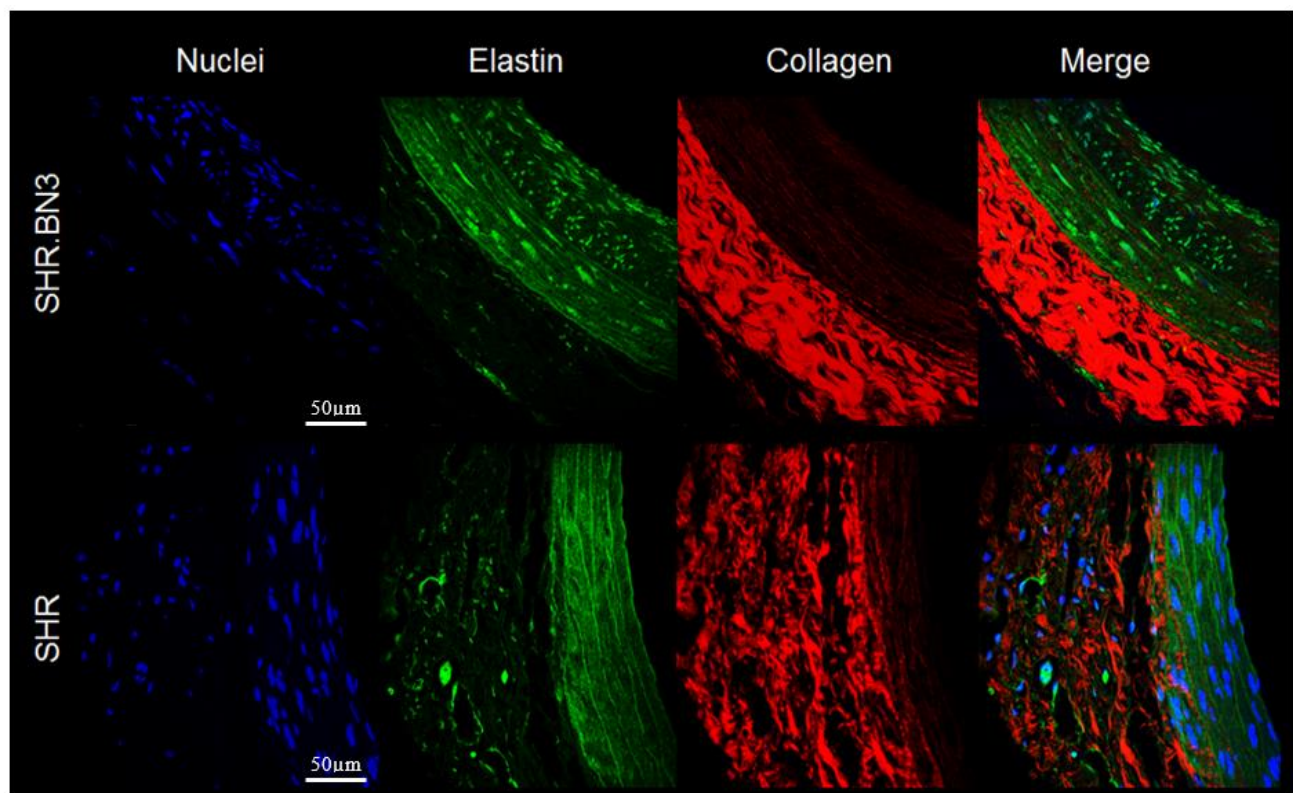


Figure 1. SHG and MPE images of the SHR and SHR.BN3 arteries 8-weeks post injury, 63X magnification.