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# Changes in Collagen Ultrastructure due to the Collagen-Binding Protein DDR1 Impacts GPVI and VWF Binding

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Collagen fibers, a major component of the extracellular matrix of blood vessels, confer structural and mechanical integrity to the vessel wall. We have shown earlier that the assembly of collagen fibers *in vitro* is regulated by collagen-binding proteins like discoidin domain receptors (DDR1 and DDR2) [1, 2]. Recently it has been shown that the DDR1 binding site on collagen overlaps with the binding site for von Willebrand Factor (VWF)[3]. VWF and platelet collagen receptor, GPVI, binding to collagen serve as major mechanisms for platelet adhesion to collagen at sites of vascular injury[4]. Therefore, this study aims to determine how (a) DDR1 changes the morphology of collagen fibers *in vivo*, and (b) how these changes alter GPVI- and VWF-collagen interactions.

Mouse aortas were isolated from 6 month old female DDR1-/- mice and their wildtype (WT) littermates according to an approved Ohio State University IACUC protocol. DDR1 expression in the vessel wall was determined by mRNA analysis and immunohistochemistry. The ultrastructure of collagen in the arterial vessel wall was determined by transmission electron microscopy (TEM) and atomic force microscopy (AFM). Aortic collagen fiber diameters were measured for the tunica media and the tunica adventitia by TEM, and the depth of D-periodicity for fibers were measured from AFM topographic images. The effect of DDR1 on GPVI or VWF binding to collagen was investigated by microplate assay where homogenized aortic collagen fibers were immobilized on microwells, incubated with GPVI or VWF, and binding was determined by immunodetection.

Consistent with previous reports[5], DDR1 was found to be expressed in smooth muscle cells of the mouse arterial vessel wall. No gross structural differences were found in the arterial vessel wall of DDR1-/- aorta compared to WT. However for both the tunica media and tunica adventitia, DDR1-/- mice had altered collagen ultrastructure compared to their WT littermates (Fig.1). Collagen fibers from DDR1-/- mice exhibited altered depth of D-periodicity in TEM images (Fig.1) and AFM topographic images. Homogenized aortic collagen from DDR1-/- and WT mice showed differences in their ability to bind VWF.

These observations translate our *in vitro* studies to *in vivo* collagen regulation by DDR1. While overexpression of DDR1 was found to reduce collagen fiber diameters, deletion of DDR1 increases collagen fiber diameter and alters depth of D-periodicity. While these changes in collagen ultrastructure can impact the structure and mechanical properties of the vessel wall, we provide an additional functional significance of expression of DDR1 in the vessel wall. DDR1 expression reduces the ability of VWF-collagen interactions and thereby inhibits platelet adhesion.

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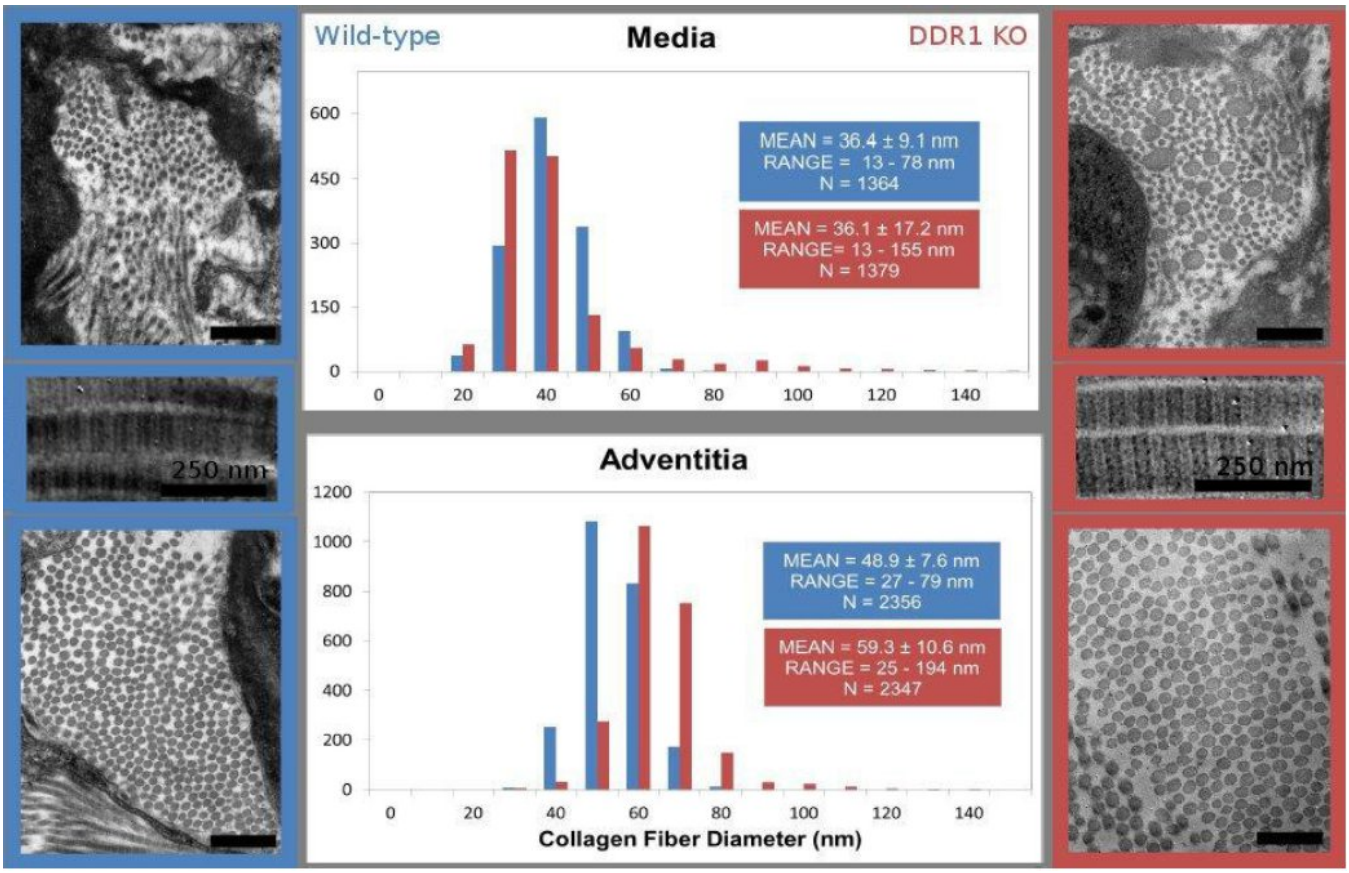


**Figure 1: DDR1 KO aortas exhibited altered collagen fiber structure compared to WT aortas.** Mouse aortas were dissected, fixed, and processed for routine TEM. (n=3 for KO; n=3 for WT) Scale bars = 500 nm, unless otherwise noted.

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