## Role of Smooth Muscle Cells in Vascular Ehlers Danlos Syndrome

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The vascular type of the Ehlers-Danlos syndrome (vEDS) is widely regarded to be the most clinically severe form of EDS due to the high risk of uterus, gut, and artery rupture. Vascular EDS is caused by mutations in the *COL3A1* gene, which instructs the body's cells how to produce a specific and necessary protein called type III collagen. Patients with vEDS possess altered genetic codes that form blueprints for structurally abnormal proteins, leading to cellular difficulties in producing type III collagen.

Normally, type III collagen is exported from inside cells to an outside environment called the extracellular matrix, which serves as a structural scaffolding to surround and support cells, tissues, and organs. Certain organs, such as the uterus, gut, and arteries, contain type III collagen to maintain their structure. Since patients with vEDS are not able to supply enough type III collagen, these same organs are prone to rupture. Medical scientists have noted that the uterus, bowels, and arteries share structural characteristics (for example, they are all hollow and expansible) that are partially dependent on properties of type III collagen, and the conventional wisdom holds that the disease largely results from a lack of collagen-mediated support. However, it is important to note that the uterus, bowels, and arteries also all depend on the contraction of special muscle cells (called smooth muscle cells, or SMCs) within the organ wall in order to function properly. **The strength generated by the contraction of these SMCs may also serve a necessary role in maintaining the integrity of these organs.**

Recent research has provided evidence that SMC function is guided by cues in the immediate environment, including those cues provided by proteins like the collagens in the extracellular matrix and the mechanical forces acting on tissues. These cues provide even more critical information in the context of organ injury and repair, described in the literature as "remodeling" of organs, since SMCs are responsible for sensing tissue damage, producing new matrix proteins to form a scar, and restoring normal contractile function once the repair is in place. The same organs affected by vEDS are also known to rely heavily on the remodeling process. For example, the pregnant uterus must reshape to accommodate the growing fetus, the gut must reshape following minor obstructions, and arteries must remodel to accommodate sudden changes in blood pressure. If these organs are not able to fully recover contractile strength after these remodeling episodes, they may suffer a localized weakness and vulnerability to rupture. **Thus, we hypothesize that vEDS may result not only from decreased support from type III collagen in the extracellular matrix but also from SMC dysfunction, especially the organ's response to injury or stress.**

To test this hypothesis, we propose the following Specific Aims:
**Specific Aim 1: Determine if there are different responses to injury of arteries that are deficient in type III collagen in comparison with arteries containing normal levels in mice**

**expressing less or normal amount of type III collagen, i.e. *Col3a1*$^{+/-}$ and *Col3a1*$^{+/+}$ mice, respectively.**

To gauge response to injury in the large arteries (the arteries that are prone to dissection and rupture in human vEDS patients), we used surgical ties to impede blood flow in the carotids of *Col3a1*$^{+/+}$ and *Col3a1*$^{+/-}$ mice, a procedure called carotid ligation. We have been able to show that the vascular SMCs in mice with less type III collagen in their arteries respond differently to this procedure (Figure 1). Very few SMCs grow into the lumen of the artery in mice with normal amounts of type III collagen (*Col3a1*$^{+/+}$ arteries, Figure 1a) while SMCs in the collagen-deficient *Col3a1*$^{+/-}$ artery form a large, eccentric mass of disorganized and "fluffy" SMCs, which reflect changes in SMC properties and growth in these arteries. **Therefore, a deficiency in type III collagen changes the way that SMCs respond to arterial injury (carotid ligation).**

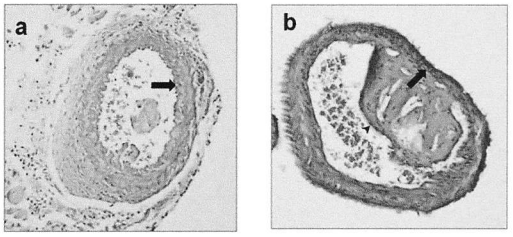


**Figure 1.** Differential response to carotid artery ligation between *Col3a1*$^{+/+}$ and *Col3a1*$^{+/-}$ mouse arteries. (a) Thickening of the wall of an injured *Col3a1*$^{+/+}$ artery little to no evidence of SMCs within the lumen of the vessel. (b) A abnormal and large eccentric mass of SMCs in the *Col3a1*$^{+/-}$ artery after carotid ligation. **These images show that the SMCs respond differently to injury when there is less type III collagen.**

We also propose similar experiments inducing injury to the gut, another organ system prone to rupture in vEDS patients. Gut-banding surgeries that mimic intestinal obstruction can be used to investigate any possible differences in bowel remodeling between *Col3a1*$^{+/+}$ and *Col3a1*$^{+/-}$ mice.

**Specific Aim 2: To confirm differences in *Col3a1*$^{+/-}$ and *Col3a1*$^{+/+}$ SMCs transferred from the mice to the culture dish in the lab by testing their ability to respond to molecular cues associated with remodeling.**

A molecule named TGF-β1, which has been identified as a master signal initiating remodeling because it is released from damaged matrix, stimulates SMCs to proliferate and produce more collagen in the short-term, and stimulates SMCs to settle and re-form their contractile machinery in the long-term. It has been suggested that the switch between the short-term and long-term behaviors in SMCs is triggered by interactions between SMCs and those extracellular matrix molecules they have recently produced. Thus, it is possible that SMCs unable to produce enough type III collagen in response to injury will later fail to achieve the levels of interactions necessary to switch their

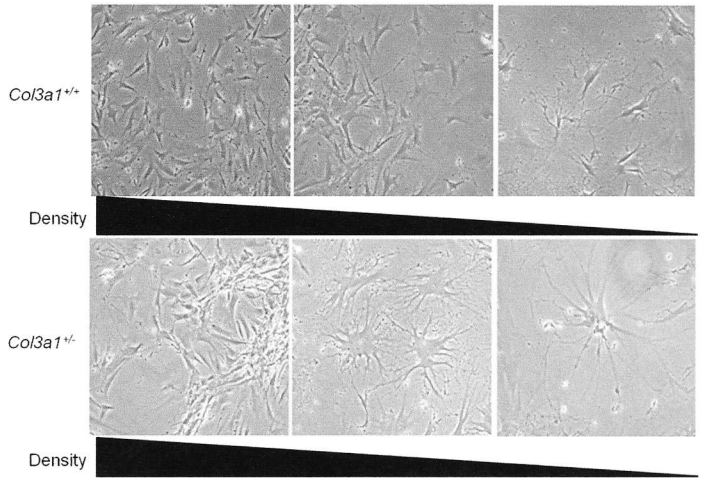


**Figure 2.** Differential cell shapes of *Col3a1*$^{+/+}$ and *Col3a1*$^{+/-}$ mouse SMCs grown in dishes. Although the cell shapes of *Col3a1*$^{+/+}$ and *Col3a1*$^{+/-}$ SMCs (top and bottom, respectively) look similarly needle-shaped at higher densities (left panels), *Col3a1*$^{+/-}$ SMCs at lower densities (middle and right panels) seem bigger and more star-shaped and lack the directionality needed for cellular contraction.

behaviors back to normal. We propose to measure the behaviors of SMCs from *Col3a1*$^{+/+}$ and *Col3a1*$^{+/-}$ mice in the culture dish following stimulation with TGF-β1, including their ability to form contractile protein machinery. We have been encouraged by early observations of *Col3a1*$^{+/-}$ SMCs in the dish showing grossly abnormal cell shapes, in comparison with *Col3a1*$^{+/+}$ SMCs (Figure 2). These data again show that the SMCs that are not making normal levels of type III collagen are different from SMCs making normal amounts of this collagen.

**Specific Aim 3: To restore the ability of type III collagen-deficient cells to respond to remodeling cues in the dish and the ability of type III collagen-deficient organs to respond to injury in mice using drugs that affect the SMCs.**

These proposed studies are focused on the changes in the SMCs when there is less type III collagen. This is because it is simpler to use drugs to change the SMC behavior back to normal than it is to replace the missing collagen. We want to use the artery injury model to determine if we could use drugs to make the SMCs respond correctly to injury. For example, the drug rapamycin has been shown to stimulate SMCs to form their contractile machinery. At the same time, this drug has already been approved for use in human patients to prevent artery lesions causing heart attacks from reforming after those blockages have been opened with metal stents. As these artery lesions are formed in large part by over-growth of SMCs in damaged vessels, this drug—in effect—is being used to prevent inappropriate SMC behavior in response to arterial injury. Our experiments would examine whether it could potentially correct the similar defect that we suspect is occurring in vEDS patients. We plan to treat our *Col3a1*$^{+/-}$ SMCs with rapamycin in the dish, to test whether this drug will stimulate the cells to form contractile machinery. We also plan to treat our *Col3a1*$^{+/-}$ mice with rapamycin before and after arterial injury, to test whether the drug may normalize the behavior of collagen-deficient SMCs in the remodeling response. If successful, this drug would be an excellent candidate for therapy in vEDS patients, to prevent aberrant remodeling and restore contractile function in response to tissue injury, possibly decreasing the vulnerability to rupture in the tissues of vEDS patients. **While restoring the structural support of type III collagen to tissues would be clinically difficult, the use of an oral drug to restore the contractile strength of SMCs could yet increase the resistance of organ walls to rupture and provide a significant leap forward in the management of vascular Ehlers Danlos syndrome patients.**