Towards Cell-Mediated Regeneration of Elastic Matrix Structures in De-Elasticized Blood Vessels

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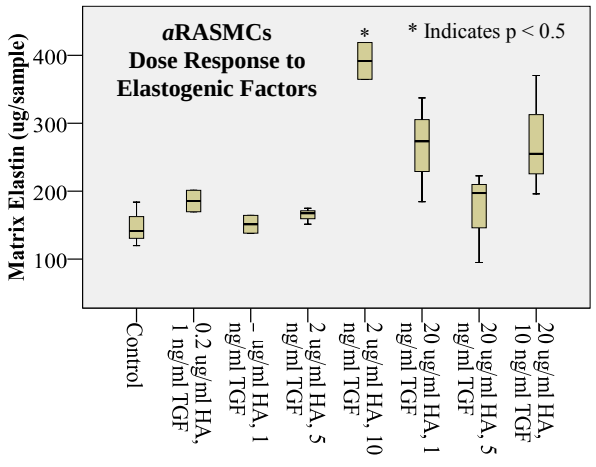
Statement of Purpose: Elastin, a major structural extracellular matrix protein, critically maintains vessel structure and regulates cell-signaling pathways involved in morphogenesis, injury response, and inflammation. One pathological outcome of elastic matrix disruption in the aorta, is the development of an abdominal aortic aneurysm (AAA), which results from proteolytic (e.g., matrixmetalloproteases; MMPs) breakdown of the elastic matrix by inflammatory cells recruited to injured/diseased vessel segments. AAAs ultimately lead to vessel wall weakening, over-expansion, and rupture. Surgical grafting at AAA sites, the primary mode of AAA treatment, can cause significant long-term complications and do not reinstate a healthy cell phenotype or matrix environment. Thus, there is a need to regenerate and stabilize elastic matrices to regress AAAs. However, such regeneration is impeded by poor elastogenicity of adult smooth muscle cells (SMCs), and the lack of tools to up regulate elastin precursor synthesis, matrix deposition, and fiber formation. Previously, we have shown that hyaluronan oligomers (HA-o) (primarily HA 4 mer; MW: 756 Da) and TGF-β1 (termed elastogenic factors) synergistically up-regulate elastin precursor (5-fold vs. non-additive) and matrix synthesis (8-fold) by healthy, adult rat SMCs, and further enhance elastin matrix yield (45 vs. 5.3%), crosslinking, stability, and fiber formation[1]. While these results greatly benefit tissue engineering elastin rich constructs using healthy SMCs, also in situ elastic matrix regeneration within AAAs demands likewise response of AAA SMCs. To assess this, AAA RASMCs (*a*RASMCs) were isolated from $CaCl_2$- induced rat AAAs, elastogenically stimulated (0.2 μg/ml HA-o and 1 ng/ml TGF-β1) in vitro over 3 weeks of culture. The cells yielded far less elastic matrix (1.7–fold) than that produced by similarly cultured healthy SMCs. Yet, a decrease in MMP production and calcification was noted, indicating restoration of a healthier SMC phenotype[2]. Following up on these results, we presently assess the dose response of *a*RASMCs to our factors. This information will guide investigations into feasibility of induced in situ elastin regeneration/ repair within AAAs.

Methods: To induce AAA formation, adult rat infrarenal abdominal aortae were treated peri-adventitially with 0.5M of $CaCl_2$ for 15 minutes. This model has been conclusively shown to induce proteolytic elastin degradation and aortal expansion similar to human AAAs[3]. Rats were euthanized, and aortae size-analyzed at 28 days post surgery. Primary *a*RASMCs were isolated from the aortal media layer using an explant technique, and cultured in basal DMEM-F12 containing 10% v/v FBS. The isolated cells were cultured for 3 weeks and their matrix production under basal conditions and when induced with TGF-β (1-10 ng/ml) and HA-o (0.2-2 μg/ml) were compared to additive-free *a*RASMCs and healthy RASMCs of identical passage (n = 3/culture). At 3 weeks, cell layers were assayed for DNA (flurometric assay of Labarca and Paigen), tropoelastin precursors, and matrix elastin (Fastin dye-binding assay). Desmosine crosslinks (ng/ng of matrix elastin) were quantified using AA analysis. Matrix ultrastructure was evaluated using TEM and matrix calcification by Von Kossa staining. Zymography quantified production of active MMPs 2, 9.

Results: Aneurysm development was confirmed by a ~45% local increase in aortal diameter at 28 days post-injury. The cells derived from injured aortae appeared to represent a mixed population with a significant number amongst them exhibiting decreased volume/spreading. Almost all the cells however expressed SMC α-actin. Cell proliferation was not significantly different with the variable doses, excluding the highest dose (20 μg/ml HA-o and 10 ng/ml TGF-β1), which resulted in a decrease (0.71± 0.08-fold vs. control *a*RASMCs; $p<0.05$). The *a*RASMCs exhibited no changes in tropoelastin production, yet a promising increase in matrix elastin (2.64 ± 0.26-fold vs. control *a*RASMCs; $p<0.05$) with the addition of an optimal dose of 2 μg/ml HA-o and 10 ng/ml TGF-β1; in this case, elastic matrix amounts were comparable to that generated by healthy RASMCs. TEM showed that elastin fiber formation was significantly enhanced in *a*RASMC cultures that received the elastogenic factors at the optimized dose, relative to control *a*RASMCs, and healthy RASMC cultures. Encouragingly, the elastogenic factors did not enhance production of active MMPs activity or calcific deposits.

Conclusions: Our outcomes provide evidence that *a*RASMCs enhance matrix elastin synthesis in response to optimized doses of elastogenic factors and can be restored to a healthier phenotype (i.e., inhibited proliferation, calcification, & MMP production).



References: [1]Joddar B et el. Biomaterials 2006. [2] Kothapalli et al. Tissue Eng Part A 2009. [3]Isenberg JC et al. Circulation 2007. **Support:** NIH grants HL 007260 (Gacchina CE), EB006078-01A1, R21EB006078-01A1, and RO1HL0092051-01A1 (Ramamurthi A).]