Elastogenic Cues Provided by TGF-β and HA Oligomers Significantly Enhance Vascular Elastin Matrix Synthesis

C. R. Kothapalli[1], P. M. Taylor[2], R. T. Smolenski[2], M. H. Yacoub[2], A. Ramamurthi[1]
[1]Clemson University-Medical University of South Carolina Bioengineering Program and Department of Cell Biology and Anatomy, Charleston, SC 29425, USA; [2]National Heart and Lung Institute, Imperial College London at Harefield Hospital, Heart Science Center, Harefield, Middlesex UB9 6JH, UK.

Introduction: The extracellular matrix (ECM) within native vessels consists predominantly of cross-linked elastin and collagen that contribute to vessel elasticity and stiffness respectively. Thus, congenital absence or disease-induced degradation of vascular elastin, its malformation within native vessels, and the limitations to it's regeneration in vascular tissue engineered constructs due to innately poor elastin synthesis by adult vascular cells can compromise vascular homeostasis [1]. To address these limitations, we seek to evaluate novel ECM components that will provide elastogenic cues and may be stabilized into a scaffolding biomaterial for elastin matrix generation *in vitro* [2]. Our recent studies have shown that hyaluronan (HA) influences the elastogenic phenotype of vascular SMCs in a fragment size-specific manner [3, 4]. Transforming growth factor (TGF-β) has also been implicated to up-regulate cellular expression of tropoelastin mRNA besides augmenting LOX-mediated cross-linking of soluble tropoelastin into a mature, insoluble matrix layer [5]. This study tests the hypothesis that concomitant delivery of TGF-β1 and HA will significantly upregulate elastin synthesis, cross-linking and fiber formation.

Methods: Adult rat aortic smooth muscle cells (RASMC) (passage 4) were seeded at $5x10^4$ cells/2.4 cm^2. HA of fragment sizes designated as HMW-HA (1500 kDa), LMW-HA (200 kDa), VLMW-HA (20 kDa) and Oligomers (0.6 kDa) were exogenously supplemented to RASMC twice weekly at concentration of 0 (control), 0.2 μg/mL (n=3/case). TGF-β (1 ng/mL) was exogenously supplemented to the cell culture wells except for controls. After 21 days of culture, the cell layers were harvested and analyzed through biochemical assays for DNA, elastin and collagen. Extracellular elastin was quantified in terms of soluble tropoelastin collected in the medium, soluble and insoluble elastin deposited as cellular matrix. Trends in soluble tropoelastin and insoluble cross-linked elastin were semi-quantitatively confirmed through western blot analysis and desmosine assay, respectively. Peptide/amino acid analysis was performed to compare cultured elastin and native rat aortic elastin. The ultrastructural organization of elastin and collagen matrix and their abundance were assessed using transmission electron microscopy (TEM) and confocal microscopy.

Results/Discussion: After 21 days of culture, cell proliferation increased 3-fold in control cultures, while TGF-β drastically inhibited cell proliferation in all the cases by 50±10% ($p<0.05$) except in the presence of HMW-HA. TGF-β also inhibited collagen production in inverse correlation to the added HA fragment size except for HA oligomers, which stimulated a dramatic 12±2 fold increase in collagen synthesis. When compared to controls, TGF-β stimulated tropoelastin production 6.5± 0.5 fold in the presence of HA oligomers, but suppressed it by 10± 3.5% in the presence of HMW-HA. No significant changes in tropoelastin production were observed in the presence of other HA fragment sizes. Addition of TGF-β alone resulted in a 3-fold increase in crosslinked (matrix) elastin formation compared to control. This was further increased 5±0.5 fold by addition of HA oligomers ($p<0.05$ in all cases vs. controls); larger HA fragments had less of an impact. Interestingly, HMW-HA suppressed the total matrix produced with respect to the control. Western blot and desmosine assays semi-quantitatively confirmed the observed biochemical trends for tropoelastin and matrix elastin, respectively. Immunocytochemistry of cell layers exhibited the abundance of elastin/collagen matrix engulfing the cells while TEM images of the matrix ultra-structure revealed the fibrillin-mediated elastin fiber deposition. Peptide/amino acid analysis demonstrated remarkable similarities between cultured elastin and native rat aortic elastin.

Conclusions: Addition of HMW-HA to TGF-β supplemented cultures inhibits cellular elastin synthesis relative to control cultures, while smaller fragments enhance elastin matrix synthesis to levels that correlate inversely to HA fragment size, with oligomers demonstrating the most dramatic increase. TGF-β significantly enhances the cross-linking of soluble tropoelastin into matrix elastin, which is one of the primary objectives of this study. The results clearly demonstrate that concomitant delivery of TGF-β in the presence of HA oligomers dramatically upregulates elastin synthesis by RASMCs. The current results are useful to establish guidelines for regeneration of biologically and structurally faithful mimics for regeneration of vascular elastin networks.

References:

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