IGF-1-Induced Elastin Synthesis and Cross-linking by Vascular Smooth Muscle Cells is Mediated by HA-Fragments

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Introduction: Though vascular tissue engineering is aimed at developing structurally and functionally-faithful vascular elastic matrices, there has been only limited progress towards regenerating these complex structures vital to tissue homeostasis. Efforts to regenerate lost elastin structures *in vivo* and within tissue engineered constructs are limited by the unavailability of scaffolds that can provide cellular cues necessary to upregulate innately poor elastin synthesis by adult vascular cells [1]. To address these limitations, we aim to explore hyaluronan (HA) based scaffolds to induce elastin matrix regeneration. HA, a GAG, has been implicated to play key role in elastin synthesis by VSMC [2]. Our recent studies have shown that HA influences the elastogenic phenotype of VSMC in a fragment-specific manner [3, 4]. Earlier investigations reported that Insulin growth factor (IGF-1) stimulates VSMC for elastin synthesis by up-regulating cellular expression of tropoelastin mRNA [5, 6]. This study tests the hypothesis that concomitant delivery of IGF-1 and HA will benefit up-regulation of elastin synthesis and elastic fiber formation.

Methods: Adult rat aortic smooth muscle cells (P4-6) were seeded at 10^5cells/2.4 cm^2. HA fragment sizes designated as HMW-HA (1.5×10^6 Da), LMW-HA (200 kDa), VLMW-HA (20 kDa) and oligomers (600 Da) were exogenously supplemented to the cultures twice weekly at concentrations of 0 (control), 0.2 µg/mL (n=3/case). IGF-1 (500 ng/mL) was exogenously supplemented to the culture wells except for non-HA, non-IGF controls. After 3 weeks 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 matrix layers. Trends for soluble tropoelastin were semi-quantitatively confirmed through western blot analysis while selected results for insoluble cross-linked elastin were confirmed through desmosine assay. 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, cell proliferation increased by 4.5±0.9 fold in control cultures, while IGF-1 inhibited cell proliferation by 50% in all the cases except in the presence of oligomers and HMW-HA ($p<0.05$). Collagen production increased 2.1±0.2 fold when VLMW-HA and LMW-HA were added in the presence of IGF-1, while addition of HMW-HA and oligomers did not have any effect ($p<0.05$ vs control). IGF-1 stimulated tropoelastin production in inverse correlation to the added HA-fragment size, except in the presence of HA oligomers, which suppressed tropoelastin expression by 20± 3%. HA fragments up-regulated cross-linked elastin matrix synthesis in inverse correlation to their fragment size (Figure 1). The addition of IGF-1 only with no HA did not upregulate insoluble elastin matrix production compared to control. IGF together with oligomers inhibited elastin matrix synthesis by 40±10%. Western blot and desmosine assays semi-quantitatively confirmed the observed biochemical trends for tropoelastin and matrix elastin, respectively. Immunoflourescence study 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.

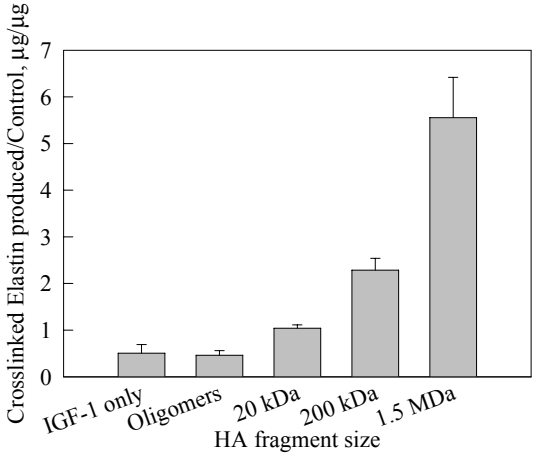

Fig 1. Crosslinked insoluble elastin matrix synthesized by RASMC at various culture conditions of HA and IGF-1.

Conclusions: Crosslinking of tropoelastin into matured elastin significantly enhanced by concomitant delivery of IGF and HA fragments, except HMW-HA and oligomers; Similar trends in collagen and tropoelastin production was observed. The results clearly demonstrate that exogenous supplementation of IGF-1 in the presence of HA fragments influence elastin synthesis by RASMC in a size-dependent manner. Current results in conjunction with the ongoing work will assist in establishing guidelines for the development of biologically and structurally faithful tissue-engineered constructs for degraded elastin matrices within damaged blood vessels and other non-vascular tissues.

References:

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