Extracellular Matrix Proteins Regulate Heart Valve Calcification

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Introduction: Tissue-engineered heart valves have the potential to circumvent problems associated with existing valve replacements. Calcification is a common cause of native valve failure, and transforming growth factor-beta1 (TGF-β1) plays a critical role in valvular disease, in part by stimulating valvular interstitial cells (VICs) to assume a pathological, activated phenotype [1]. Biomaterial properties alone can induce increased endogenous TGF−β1 production, leading to pathological valve remodeling [1]. Extracellular matrix (ECM) components are widely used either alone or in combination with other materials in the creation of scaffolds for engineered valves, and identification of ECM components that promote VIC resistance to dysfunction may directly impact the design of biomaterials engineered heart valves. In the current research, we have found that the propensity of VICs to assume a diseased phenotype is dependent upon the characteristics of the biomaterial, with both integrin activation and TGF-β1 sequestration playing important roles in protecting VICs against TGF-β1-induced valve disease.

Methods: VICs were cultured in low-serum (1% FBS) medium on tissue culture polystyrene (TCPS) that was coated with collagen type I (2 μg/cm^2), fibronectin (FN, 5 μg/cm^2), laminin (LN, 2 μg/cm^2), or left uncoated, either in the presence or absence of 0.5 ng/ml TGF-β1. In order to investigate the role of integrin interactions with the ECM in the process of VIC calcification, VICs were also pre-treated with specified peptide sequences – GRGDS, GRGDTP, or DGEA (all peptides delivered at 1 mM in solution) – prior to seeding on unmodified TCPS surfaces. After five days of culture, cell morphology, α−SMA and TGF-β expression, and calcific nodule formation were assessed. Calcific nodules were positively identified via staining with Alizarin Red S, while standard immuno-fluorescent staining was performed to visualize α-SMA (a marker of VIC activation/dysfunction), TGF-β1, and cell morphology. Lastly, the potential for calcification regulation via TGF-β1 sequestering by different ECM components was investigated by incubating ECM-coated wells with 0.5 ng/ml TGF-β1, rinsing extensively to remove unbound TGF-β1, then quantifying bound TGF-β1 via a fluorescently-linked enzyme immunosorbent assay [2].

Results/Discussion: As shown in Figure 1, VICs cultured on unmodified TCPS readily calcify, both in the absence and presence of exogenous TGF-β1, while VICs cultured on Coll or FN form significantly less calcific nodules with either TGF-β1 treatment condition ($p<0.01$). The results in Figure 1 clearly demonstrate that VIC calcification, morphology, and α-SMA expression are highly dependent upon the type of protein on which they are cultured.

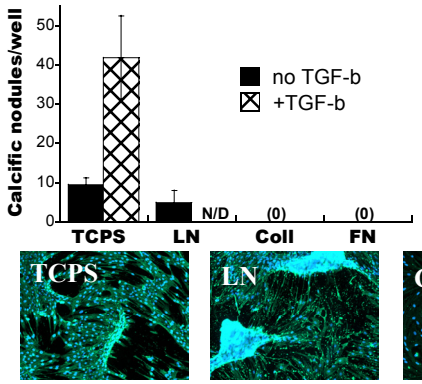

Figure 1. Top: calcific nodule formation by VICs cultured on TCPS or various ECM proteins. Bottom: staining of VIC cultures for α-SMA.

As shown in Figure 2, neither RGDS nor RGDT treatment had a substantial effect on VIC calcification. However, both the pre-treatment with DGEA and culture on DGEA-treated surfaces significantly reduced VIC calcification, even in the presence of TGF-β1. These results implicate the DGEA receptor ($\alpha_2\beta_1$ integrin) as a regulator of VIC calcification.

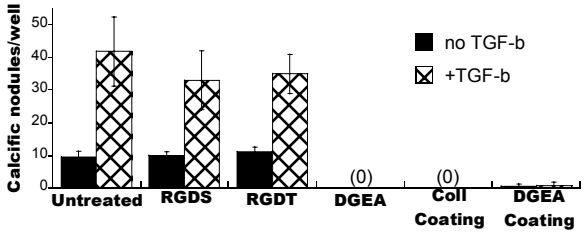

Figure 2. Impact of specific peptides on VIC calcification.

The binding of TGF-β1 by FN and Coll-coated surfaces (shown in Figure 3) may also participate in the regulation of TGF-β1-induced VIC calcification via the sequestration of TGF-β1.

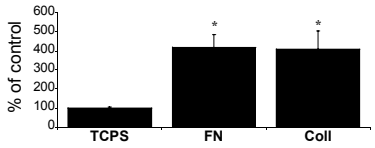

Figure 3. Amount of TGF-β1 sequestered by TCPS and ECM-coated plates. *$p<0.05$

Conclusions: These results point to an important role of ECM proteins in the regulation of VIC dysfunction. We have identified the $\alpha_2\beta_1$ integrin receptor and the ability of various ECM components to bind TGF-β1 as playing critical roles in VIC calcification; current research focuses on the translation of these findings to the design of biodegradable hydrogels for valve tissue engineering. The design of biomaterials for valve tissue engineering, the elucidation of valvular disease mechanisms, and the treatment or prevention of valve calcification may benefit from these discoveries and the continued investigation of ECM regulation of valve function.

References: [1] Walker GA, Masters KS, Shah DN, Anseth KS, Leinwand, LA. *Circ Res.* 2004; 95: 253-260. [2] Cushing MC, Liao JT, Anseth KS. *Matrix Biol.* 2005; 24: 428-437.