FIBROBLASTS ENHANVE CAPILLARY NETWORK FORMATION VIA VEGF-DEPENDENT MECHANISM

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ABSTRACT
Engineering

Background. In order for the heart to function properly, there must be ample blood supply to the tissues. However, after experiencing trauma such as myocardial infarction, the heart muscle undergoes a series of responses, beginning with inflammation and ending with the formation of scar tissue. This scar tissue does not possess sufficient microvascular networks to allow the heart to function at normal capacity. Cardiac tissue engineering studies have demonstrated the importance of the angiogenic environment for revascularization. Fibroblasts (FBs) play a key role in cardiac remodeling by mediating extracellular matrix (ECM) deposition and growth factor expression, but the role of endothelial-fibroblast interactions in revascularization is poorly understood. Recently, fibroblasts have been shown to promote capillary-like network formation by capillary endothelial cells (ECs) in vitro. In addition, fibroblasts are the source of several angiogenic growth factors, including vascular endothelial growth factor (VEGF), which is the major growth factor regulating activation of endothelial cells and capillary formation *in vitro* and *in vivo*. In previous studies, we have developed a novel angiogenic scaffold which promotes angiogenesis and EC survival in vitro and enhances VEGF expression by FBs. This study tested the hypothesis that fibroblasts mediate capillary network formation by microvascular endothelial cells via VEGF-dependent paracrine mechanism.

Methods. ECs were stained with CellTracker Orange (Molecular Probes) and seeded with and without FBs on a self-assembling peptide nanoscaffold in the presence of 10% FBS, but in the absence of external growth factors (total of 10^5 cells/cm^2, 1:1 EC:FB ratio in co-cultures). At 6 or 24 hrs after seeding, samples were fixed in 2% formaldehyde, stained with DAPI to visualize cell nuclei, and fluorescent images of capillary-like networks formed by ECs were taken. The characteristic network size was determined using correlation analyses and custom-written MATLAB code. In order to study the effect of VEGF on endothelial network formation, some cultures were treated either with VEGFR-2 inhibitor, which blocks VEGF receptor 2; soluble VEGFR-1, which binds directly to VEGF in the medium; or with both inhibitors (n=3 samples in each group).

Results. In the absence of inhibitors, capillary networks formed significantly faster and were significantly larger when FBs were seeded together with ECs, as compared with EC-only cultures ($p<0.05$, ANOVA). For example, at 24 hours, the characteristic network size in EC-FB co-cultures without added inhibitor was 13.84±1.48 microns, which is significantly larger than the 7.06±0.42 microns measured in EC only cultures ($p<0.05$, ANOVA). Interestingly, co-culture images at 24 hours show a coordinated migration of both ECs and FBs into the networks, as seen by co-localization of ECs and FBs in the network structures and absence of cells of both types outside the structures. In EC-FB co-cultures, inhibition of VEGF by either VEGFR2 inhibitor or soluble VEGFR1 resulted in significantly smaller characteristic network sizes at 24 hours ($p<0.05$, ANOVA) and this decrease in network size was consistent among the different inhibitor groups (8.55±0.48 microns for VEGFR-2 inhibitor, 7.36±1.64 microns for soluble VEGFR-1), with no indication of inhibitors acting synergistically (network size of 6.68±1.33 microns for both inhibitors present). Our results demonstrate that blocking VEGF signaling by either VEGFR2 inhibitor, soluble VEGFR1, or both resulted in complete inhibition of FB-induced increases in capillary network size at both 6 and 24 hours (no difference with EC-only controls, $p>0.05$, ANOVA). Furthermore, in EC-only cultures, VEGF inhibition had no significant effect on network size at 24 hours.

Discussion. The data show that capillary network formation by microvascular endothelial cells is enhanced in the presence of fibroblasts, and that this effect is mediated by VEGF. Results show that inhibition of VEGF signaling in endothelial cells by either blocking VEGFR2 receptor or sequestering soluble VEGF from the medium effectively negates FB-induced enhancement of network formation. These results indicate that it is VEGF's interaction with its cellular receptors, and not simply activation of these receptors, that promotes network formation. This investigation advances our understanding of the angiogenic process in tissues such as the heart and contributes towards the development of vascularized scaffolds for tissue engineering applications.