A Collagen Matrix for Cell Therapy Improves the Viability, Perfusion, and Function of Infarcted Hearts through an Integrin and ILK Pathway

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Introduction:

Regenerating the heart remains the ultimate, yet elusive, goal for treating patients with advanced ischemic or idiopathic cardiomyopathy. Clinical cell therapy trials have produced mixed results, revealing modest functional improvements at best. It is believed that the recipient environment may be inadequate for retention of a sufficient number of donor cells to allow for the desired effect. To improve cell therapy, we tested echo-guided intramyocardial delivery of circulating angiogenic cells (CACs) within a collagen matrix, in a mouse model of myocardial infarction (MI). We examined the potential for the matrix to enhance cell retention, as well as myocardial perfusion, viability, and function. Since integrins are important for mediating interactions between a cell and its environment, we examined the role of integrin signaling, through integrin-linked kinase (ILK), in mediating the effects of CAC+matrix therapy.

Materials and Methods:

Seven days after left anterior descending coronary artery ligation, female C57BL6/J mice were randomly allocated to receive one of four treatments: CACs (n=29), collagen matrix (n=19), CACs+collagen matrix (CAC-matrix; n=29), or PBS (n=15). CACs were green fluorescent protein $(GFP)^+$ marrow-derived cells from male C57BL/6-Tg(CAG-EGFP)1Osb/J mice. ^{13}N-ammonia and ^{18}F-FDG PET imaging (on randomly selected mice), as well as echocardiography (on all mice) were performed at the time of treatment (baseline) and after 3 wks (follow-up). Hearts were harvested for immunohistochemistry (examination of transplanted cell retention, LV mass preservation, and arteriole density), Western Blot (ILK expression) and q-PCR (Y chromosome copy number) analysis. Furthermore, a profile of integrin expression on CACs revealed an up-regulation of the $\alpha1\beta2$ integrin receptor (a major type I collagen receptor). Therefore, in an additional set of experiments, $\alpha1\beta2$ was blocked in CACs and ILK expression (*in vitro*) and the therapeutic effects (*in vivo*) of the blocked cells were assessed.

Results:

Follow-up ejection fraction (EF) was greater in the CAC+matrix group (EF=56±2%) compared to all other groups (≤40±2%; $p<0.001$). PET analysis showed improved viability and perfusion (by 35% and 29%, respectively; $p\leq0.05$) only after treatment with CAC+matrix. Histology showed an anterior (infarct) to posterior (intact) LV wall thickness ratio of 0.7±0.1 in the CAC+matrix group, which was greater than all other groups (≤0.3±0.0; $p<0.001$). More arterioles were detected in hearts injected with CAC+matrix (10.9±1.1 per field-of-view (FOV)) compared to the other treatments

(≤6.2±0.5; $p<0.001$). Q-PCR analysis for the Y chromosome revealed a 8.6±1.4 fold increase in retention of transplanted cells in the CAC+matrix group relative to the CAC group ($p=0.001$). ILK expression was higher in hearts treated with CAC+matrix (1.4±0.1 fold) or matrix (1.6±0.1 fold) compared to hearts treated with CACs or PBS ($p\leq0.02$). We also showed that blocking the α1β2 integrin results in ILK down-regulation (1.3±0.04 fold reduction; $p=0.003$) in CACs before injection. The α1β2 block abrogated the therapeutic effects of CACs delivered within a collagen matrix (baseline EF=41±3%; follow-up EF=42±3%). Despite normal cell viability upon treatment delivery, Y-chromosome q-PCR showed negligible cell retention 3 weeks later. Arteriole density in the blocked-CAC group (3.7±0.2/FOV) and blocked-CAC+matrix group (3.5±0.5/FOV) was not significantly different. Finally, the anterior to posterior wall thickness ratio was 0.3±0.0 in the blocked CAC+matrix group, which was similar to the hearts treated with CACs only.

Discussion:

The collagen matrix enhanced transplanted cell retention and supported higher vascular density in the infarcted myocardium. Also, the combined CAC+matrix therapy preserved LV wall mass, and enhanced myocardial function, perfusion and viability. While delivery matrices have shown promise for enhancing cardiac cell therapy, the effects and mechanisms responsible remain largely unexplored. The present work provides some mechanistic insight into the effects that CAC-matrix interactions have on improving cell therapy in a mouse MI model. Blocking the α1β2 integrin receptor resulted in ILK down-regulation in CACs and this was associated with a significant reduction in the therapeutic benefits conferred from CAC-matrix treatment. This study suggests a pivotal role for ILK in the CAC-collagen matrix interaction which has shown promise as a therapeutic approach to improve function of the infarcted heart.