Integration of Cysteine-rich Angiogenic Inducer 61 into Collagen Matrix Promotes the Therapeutic Potential of Circulating Angiogenic Cells

McNeill, B; Vulesevic, B; Ruel, M; Suuronen, EJ
University of Ottawa Heart Institute, Ottawa ON; Department of Cellular & Molecular Medicine, University of Ottawa, Ottawa ON

Introduction

Myocardial infarction (MI) occurs when blood flow to the left ventricle of the heart becomes blocked. The resulting lack of oxygen results in myocardial cell death, tissue remodeling and the formation of a scar. These post-MI events significantly attenuate cardiac function and output that can eventually lead to heart failure and death. Cell therapy using circulating angiogenic cells (CACs) has emerged as a promising approach to treat MI, as they can differentiate into mature endothelial cells and secrete proangiogenic cytokines needed to promote revascularization. Results from animal models and clinical trials have demonstrated the potential for CAC therapy but the benefits remain modest due, in part, to low cellular retention and engraftment. To overcome this hurdle, a collagen-based matrix has been developed to deliver and promote CAC function. The aim of this study was to modify the matrix to further improve the therapeutic potential of CACs. We cultured CACs on the collagen matrix and analyzed the expression profile of integrins to identify which of these cell surface adhesion proteins may be present on CACs. The goal was then to modify the matrix to include proteins or motifs that will target these integrins to promote CAC retention and function. We hypothesize that the addition of an extracellular membrane protein Cysteine-rish angiogenic inducer 61 (CYR-61), an integrin associated protein, will enhance the collagen matrix and functionally improve the therapeutic potential of CACs.

Materials and Methods

Cell Isolation: Blood sample procurement was approved by the Human Research Ethics Board of the University of Ottawa Heart Institute with informed consent obtained from all participants. Mononuclear cells from human peripheral blood were isolate using Histopaque 1077 density centrifugation and CACs were enriched during a 4-day fibronectin culture. CACs were lifted and re-plated on fibronectin or on a type I collagen-based matrix. RT-qPCR: mRNA expression of 18 α- and 8 β-integrins were analyzed from the highly potent pro-angiogenic $CD34^{+}$ subpopulation of CACs purified by fluorescence-activated cell sorting. Functional assays: CACs treated with and without CYR61 were assayed for adhesion, migration, proliferation and angiogenic potential. Hindlimb ischemia model: Procedures were performed with the approval of the University of Ottawa Animal Care Committee and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The left proximal femoral artery was ligated under 3% isoflurane. Ligation and subsequent recovery was assessed using Doppler. Biomaterial preparation: Collagen I and chondroitin sulfate-C were blended on ice, cross-linked using glutaraldehyde, and quenched using glycine. CYR61 was immobilized to the matrix by EDC/NHS cross-linking.

Results

mRNA expression of integrins α5, α7, αM, αV and β3 was significantly up-regulated by 56±5.5, 60±6.4, 15±4.2, 55±4 and 67±7.5 fold respectively in $CD34^+$ cells cultured on collagen vs. fibronectin, while integrins α3 and β7 were down-regulated by 30±4.5 and 58±6.8 fold, respectively (all $p<0.05$). Since αV, β3 and αM interact with CYR61, we examined the functional response of collagen cultured CACs to CYR61. Adhesion of CACs to matrix+CYR61 was significantly increased 2.2±1 fold ($p=0.032$) over matrix lacking CYR61 and 4.8±2.4 fold ($p=0.015$) over fibronectin. Furthermore, the number of $CD34^+$ and $CD133^+$ cells was increased 3.3 and 2.7 fold, respectively, on matrix+CYR61 compared to control biomaterials. Using CYR61 as a chemoattractant, CAC migration was increased 5±2.1 fold ($p=0.036$) compared to control media without CYR61. CACs pretreated with CYR61 1h prior to an angiogenesis assay incorporated more readily into tube-like structures, 4.1±1.6 fold ($p=0.028$) more than CACs from collagen matrix and 7.3±1.4 fold ($p=0.019$) more than CACs from fibronectin. *In vivo*, transplantation of matrix-cultured CACs pretreated for 1h with CYR61 resulted in a greater perfusion recovery in a hindlimb ischemia mouse model over injection with either matrix-cultured CACs ($p=0.029$) or PBS ($p=0.0005$).

Discussion

We demonstrate that the regulation of integrins is significantly altered when culturing CACs on a collagen biomaterial. Understanding which integrins are expressed under these conditions helped identify CYR61 as a potential protein to improve our collagen matrix by enhancing endogenous and *in vitro* expanded CACs retention and function. CYR61 has the ability to act as a chemoattractant, angiogenic stimulator and can promote adhesion by interacting with the collagen-cultured CACs. These findings demonstrate a novel mechanism which may be used to promote reperfusion in ischemic tissue.