Inhibition of delta protein kinase C (δPKC) and activation of epsilon PKC (εPKC) have both been shown to limit damage from ischemia and reperfusion (I/R). Studies have been hindered by the need to use exogenous protein expression in rat cardiac allografts. Methods: Epsilon PKC activator (ψε) and PKC inhibitor attenuates the I/R injury in the transplanted heart has not been determined. We investigated the ability of PKC regulator to reduce I/R injury with prolonged ischemia in rat cardiac allografts. Results: Methods: Hearts of rat heart (RAT1) were heterotopically transplanted into A-immune, A-TNFα, and A-COX-2 deficient recipients. The recipient heart, the ascending aorta was ligated, and 2 ml of ψεPKC activator (ψεRACK, 1.5 mM) solution was then injected (ante-grade coronary injection). Hearts were procured and submerged in the same drug solution (0.5 mM) for 30 or 120 min at 4°C. Before reperfusion, 1 ml of εPKC inhibitor (ψε-1, 30 nmol) solution was injected into the recipient IVC. Control animals were treated with normal saline. Gaits were procured after 4 h of reperfusion (n = 6 each group) and analyzed for superoxide generation by the spin-trapping method; for myeloperoxidase (MPO) activity, TNFα, IL-1β, and MCP-1 production by ELISA; and for apoptosis by TUNEL and by caspase-2, -3, -8, and -9 activities. Results: With 30-min ischemia, MPO activity, TNFα production, and caspase-9 activity decreased significantly in the PKC-regulated groups. With 120-min ischemia, MPO activity, TNFα, IL-1β, MCP-1 production, cardiomycocyte apoptosis, and caspase-2, -3, -8 and -9 activities decreased significantly in the PKC-regulated groups. Superoxide activity and caspase-8 activity did not differ significantly between the two groups with 30-min or 120-min ischemia. Conclusions: Combined treatment with ψεPKC activator and εPKC inhibitor alleviates the I/R injury that occurs with prolonged ischemia but does not suppress generation of super oxygen and caspase-8 activity that occurs with prolonged ischemia. These peptides may be useful in clinical transplantation for organ preservation and prevention of I/R injury.
Human Fat Tissue-Derived Stem Cells Show Cardiomyocytic Differentiation After Coculture
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Introduction: Cells isolated from subcutaneous fat tissue (SFT) can give rise to cells from all three germ layers. So far differentiation into a cardiomyocyte (CM) lineage by means of co-culturing has not been shown for cells isolated from human SFT. The accessible and obtainable cell numbers would make SFT an excellent source of cells for tissue repair after myocardial infarction. Methods: CMs from neonatal rat hearts were isolated and cultured on day 1 in vitro, digested with Collagenase (10mg/ml) and cultured under standard conditions. SFT from patients undergoing liposuction was collected and digested with Collagenase for 90 minutes to obtain a single cell suspension. Cells were plated under standard conditions and transfected with a lentivirus encoding eGFP. SFT cells were then co-cultured with the neonatal rat CM in a 1:1 ratio. FACS analysis of adherent SFT cells was done at day 1 and 5 prior to coculture for CD34, CD45, CD105, and CD117. Immunohistochemistry of cardiac specific Titin, Troponin T and eGFP of co-cultured cells was done after 14 days or total RNA was harvested and an RT-PCR was performed. Results: More than 1.3x10^6 cells/cellgram could be isolated routinely from SFT and 30% did adhere to tissue culture plastic. A FACS analysis of the adhering SFT cells showed an expression of CD34 (48% ± 6) and CD45 (6% ± 2) after 1 day in culture which dropped to undetectable levels after 5 days. CD105 expression was 50% (+/-8, day 1) which dropped to undetectable levels after 5 days (+/-2, day 6). CD117 could not be detected at any time. The cocultures were dispersed after 14 days and re-plated. Immunohistochemistry staining showed dual positivity of SFT for eGFP and Titin but not for Troponin T. This was confirmed in the RT-PCR reaction with human specific Primers for Titin that do not cross react with the rat CM. The RNA expression level in the CM and SFT coculture was 11 fold above control (corrected for GAPDH). Conclusion: SFT cells show the predominantly cardiomyocytic phenotype in vitro. The properties that determine the severity of dysfunction are not fully understood. Using a finite element model, we sought to better define the relative contributions of passive material properties (stiffness) versus active properties (contracting myocytes) in determining regional wall motion. We tested the hypothesis that in order for a segment to be akinetically and not dyskinetic, it must contain contracting myocytes: Methods: Using two-dimensional echocardiographic images from a repurposed ovine anterolateral infarct, we developed a three-dimensional finite element mesh of the left ventricle. The model describes both diastolic material properties defined by a constant, C, and systolic myocardial material properties defined by an isometric tension, T0,M. A reduction in the ability of the infarcted region to develop active stress was accomplished by scaling the parameter T0,M to represent the percentage of contracting myocytes between 0% and 100%. The simulated change in wall thickness between end-diastole and end-systole was measured using the fractional change of average radial strain (RS). Akinesis was defined as an average RS between -0.01 and +0.01, where a more positive or negative value represents hypokinesis or dyskinesis, respectively. The diastolic and systolic properties necessary to produce akinesia were determined using an iterative process. We then modeled an infarct with no contracting myocytes and increased the diastolic stiffness required for akinesia until RS fell between -0.01 and +0.01. Results: As stiffness, C, increased from normal (0.876 kPa) to ten times normal (10 kPa), the percentage of T0, M necessary to achieve RS between -0.01 and +0.01 increased from 20-50% . When the percentage of contracting myocytes was assumed to be zero, a stiffness of C=250 kPa was necessary. Conclusions: In this finite element model of left ventricular myocardial infarction, if wall stiffness is less than 250 times normal, the presence of contracting myocytes in the infarct zone is necessary to prevent dyskinetic wall motion. Nonviral FasL Protects Allogeneic Myoblasts Against Cell Death in Cardiomyogenesis
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Background: FasL, which promotes T-cell apoptosis through interaction with the death receptor Fas, has been proposed to immunoprotect allografted cells. However, success with this strategy has been limited in part due to inflammatory side effects of viral vectors. We hypothesize that overexpression of FasL in myoblasts via a non-viral vector will provide protection of allografted cells in heart. Methods: C2C12 myoblasts were stably transfected with FasL and co-cultured with activated T-cells. T-cell apoptosis was examined by annexin V expression. In vivo, control animals received untransfected DAPI-labelled myoblasts (2x10^6), while treated animals (FasL group) received transfected labeled myoblasts where 25% expressed FasL. Myoblasts were injected into cryoinjured myocardium, the kidneys or subcutaneously in each case. The transgene was evaluated post-mortem and engrafment by histology at 1 hour and 3, 10 or 14 days post-injection. Results: FasL transfected myoblasts induced a fourfold higher apoptosis rate in T-cells than untreated myoblasts in vitro (32.6±2.5% vs 7.0±3.9%, P<0.001). In vivo, FasL protected allogenic cells after injection into cryoinjured myocardium up to 14 days (21±8±1/HPF vs 8±9±1 control, P<0.05). Concurrently, the maximum infarct diameter was reduced (0.25±0.02 vs 0.29±0.03 mm; P<0.05) and infarct wall thickness was increased (0.11±0.03 vs 0.07±0.03 mm; P<0.05). Similarly, FasL protected allogenic cells at 1h, 3d and 10d after subcutaneous injection as compared to controls (25±6±0, 232±7±1 and 231±6±/HPF for FasL vs. 49±2±6, 0 ±0 HPF for controls, P<0.001). Surprisingly, FasL did not protect allogenic cells after injection under the kidney capsule. Conclusion: Functional FasL, delivered non-virally, can more than double survival of allogenic cells after transplantation into injured myocardium or subcutaneously. FasL might be a useful tool in allogenic cell therapy. Cyclic Stretch of Adult Human Mesenchymal Stem Cells Induces Expression of Early Cardiac and Neurogenic Genes
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Background: Human mesenchymal stem cells have shown to be plastic and amenable to transformation into cardiomyocytes. Myocardial regeneration using direct injection of stem cells into injured myocardium results in low yield of differentiated cardiomyocytes. Alternatively, stem cells have been preprogrammed using chemicals, growth factors, and cytokines before using them in coculture of cells into cardiomyocytes in vitro. We explored the role of cyclic stretch on human mesenchymal stem cells (hMSCs) as a stimulus to promote transdifferentiation. Methods: hMSC were cultured in 6 well plates and subjected to programmable square cyclic stretch at 1 Hz using the Flexercell system with 4% and 8% elongation of cells alternating every 12 hours for 7 days in a humidified incubator with 5% CO2 at 37°C. Total RNA was isolated from the stretched hMSC and unstretched control experiments using microarray (Hu133A gene chips, Affymetrix Inc) and lineage specific gene expression was studied. Genes were profiled in triplicate for cardiogenic, adipogenic, neurogenic, osteogenic and skeletal lineage. Results: The data was normalized and lineage specific genes were analyzed using Gene Spring V5.0. Induction of synaptobrevin, macrotubule associated protein (MAP-2), and myocardin/bricabrains genes implicating early neurogenic lineage was observed. In addition there was also concurrent expression of Connexin43 and BMP-2 indicative of early cardiac lineage. In contrast, there was no expression of adipogenic, osteogenic and skeletal genes. Conclusions: These data suggest that adult human bone marrow stem cells are plastic and mechanical stretch bioengineers hMSC transdifferentiation to early cardiac and neurogenic lineage. This technique of preprogramming stem cells in vitro could potentially be used to increase high yield of lineage specific cells before cellular cardiomyoplasty. Cultured Adipose Tissue-Derived Stromal Cells Express the Stem Cell Marker CD24 and Show Evidence of Differentiation Into a Cardiomyocyte Phenotype
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Background: Autologous cell therapy using pluripotent cells for cardiac repair and regeneration is an attractive option in patients with cardiac dysfunction. However, left ventricular myocardial infarction is limited by the fact that the required cell numbers often exceed the number of available pluripotent cells. We have recently shown that a substantial proportion of non-adipocyte stromal cells in adipose tissue express the stem/progenitor cell marker CD24. We therefore examined whether adipose stromal cells (ASCs) could retain this marker in culture and whether they could differentiate into a cardiomyocyte phenotype in vitro. Methods: Subcutaneous adipose tissue biopsies or lipospires were obtained from volunteers. The stromal fraction cells were cultured in EBM-2 or EGM-2 medium (Clonetics-Cambrex). The expression of CD34 was assessed on days 2, 4, 6 and 8 by flow cytometry. In this finite element model of left ventricular myocardial infarction, if wall stiffness is less than 250 times normal, the presence of contracting myocytes in the infarct zone is necessary to prevent dyskinetic wall motion. Results: The expression of the stem/progenitor cell marker CD24 on human ASCs was present at consistently high levels during the first week of culture (Day 2: 95%, Day 4: 91%, Day 6: 89%, Day 8: 77%). Multiple ASCs showed evidence of spontaneous beating.