Drosophila melanogaster possess a simple linear heart tube and constitute an excellent genetic model system with which to investigate the effects of cardiomyopathic mutation. The Mhc5 myosin heavy chain mutation is located in the 'transducer'domain and elicits hypercontractile function at the molecular level characterized by high ATPase activity and enhanced in vitro motility properties. Additionally, its expression impairs diastolic relaxation of the cardiac tube reminiscent of restrictive cardiomyopathy in humans. We have investigated the effect of the Mhc5 mutation on cardiac structure/function by quantitative proteomics using isobaric tags for relative quantification (iTRAQ). Excised fly hearts from yw (control) and Mhc5 strains were digested with trypsin, reduced, alkylated and labeled with ITRAQ reagent. Peptides from each pool were mixed together prior to fractionation by strong cation exchange chromatography and subsequent reversed-phase HPLC coupled to tandem mass spectrometry. This approach identified approximately 600 proteins, of which 94 were upregulated and 86 were downregulated in Mhc5 hearts relative to yw hearts ($p<0.05$). Ontological cluster analysis of the genes encoding the regulated proteins revealed that myofibrillar disarray in Mhc5 hearts likely stems from overexpression of actin with concomitant reduction of myofibrillar assembly proteins such as spectrin, and other actin-binding proteins. Structural remodeling was also characterized by increased expression of extracellular matrix proteins. Upregulation of proteins involved in mitochondrial oxidative phosphorylation and fatty acid catabolism suggests further bioenergetic remodeling. The proteomic, structural and ultrastructural data are consistent with a model whereby the elevated ATPase activity caused by Mhc5 mutation increases energetic demand, thereby stimulating a concerted compensatory metabolic response to maintain energetic homeostasis. Ongoing protein-network/interactome analysis will help to further refine the model.

3733-Pos

Mesenchymal Stem Cells Protect Cardiomyocytes

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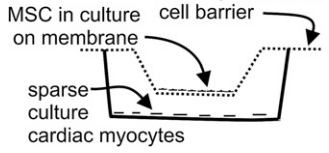
Possible therapeutic benefits of stem cell treatments have been widely investigated recently. We have presented initial reports that co-culturing mesenchymal stem cells (MSC, Lonza) with rat heart cells in primary culture can prevent the consequences of the treatment with a inflammatory bacterial endotoxin (LPS, Lipopolysaccharide-A). We now investigate how the MSC produce their beneficial actions. Using sparse primary cultures of neonatal rat ventricular or adult rat ventricular myocytes with either MSC or control cells (fibroblasts), we examine cardiac Ca^{2+} signaling. LPS causes Ca^{2+} signaling anomalies which include delayed afterdepolarizations (DADs) and Ca^{2+}-enhanced early afterdepolarizations (EADs). We find that co-cultures with cells co-mingled can prevent the untowards actions of LPS on the cardiac myocytes. The negative consequences of LPS are alterations in the normal $[Ca^{2+}]_i$ transient that is stimulated by field shocks as described above. Since the benefit of MSC co-culture are found even when a solute permeable / cell impermeant membrane separates the MSC from the LPS treated cardiac myocytes, we conclude that a paracrine action of the MSC can account for the treatment attributed to the MSCs. We continue to investigate possible beneficial signaling pathways that may explain the paracrine effect of MSCs.



3734-Pos

Mechanical and Biochemical Characteristics of Human Stem Cell-Derived Cardiomyocytes

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Cell-based cardiac repair following myocardial infarction has gained considerable interest recently, and the human pluripotent stem cell is an attractive cell source due its efficient differentiation into immature but functional cardiomyocytes. We examined the biophysical characteristics of cardiomyocytes generated from human embryonic stem cells (hESC-CMs) by measuring calcium transients, single cell contractions, and actomyosin interactions via flash photolysis. Furthermore, we compared these characteristics with those obtained from a second promising but still poorly characterized cell type, the human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM). We hypothesized that understanding fundamental biochemical and mechanical characteristics of these cells would provide insight into potential strategies to induce further cell maturation *in vitro*.

Our results suggest that hESC-CMs and hiPSC-CMs exhibit spontaneous contractions and calcium transients with similar kinetics, including time to peak $[Ca^{2+}]_i$ (116 ± 34ms vs. 155 ± 40ms) and time to 50% $[Ca^{2+}]_i$ decay (352 ± 87ms vs. 296 ± 49ms). Furthermore, quantitative videomicroscopy of resulting single cell contractions suggests that cardiomyocytes from both sources demonstrate similar resting cell size (17.1 ± 1.4um vs. 16.5 ± 4.6um), contraction amplitude ($4.2 \pm 1.6\%$ vs. $4.4 \pm 2.1\%$), time to peak contraction (0.346 ± 0.135sec vs. 0.339 ± 0.214sec), maximum contraction velocity (6.34 ± 3.50um/sec vs. 7.46 ± 4.81um/sec), and maximum relaxation velocity (3.21 ± 2.49um/sec vs. 3.40 ± 2.49um/sec).

We have also successfully isolated and purified 20 ug of myosin per million hESC-CMs. Using flash photolysis to liberate ATP in a solution of actomyosin, we have shown that the myosin binds actin and is dissociated from the complex by ATP with the expected 2nd order rate constant (~1 $uM^{-1}sec^{-1}$).

In summary, the contractile properties of hESC-CMs and hiPSC-CMs are similar to each other but differ from values published for adult human cardiomyocytes, suggesting that they are functionally immature and may benefit from *in vitro* maturation efforts.

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3735-Pos

Cell-Seeded Fibrin Scaffolds for Cardiac Tissue Engineering

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Cellular cardiomyoplasty to replace non-functional tissue following cardiac infarction appears clinically viable. Current strategies utilizing direct injection of cell suspensions are limited by low cell retention, poor cell localization, and high cell death. Synthetic biomaterials developed to enhance cell delivery can lead to problems with immune rejection, degradation, and mechanical mismatch, preventing functional integration of constructs with host myocardium. The goal of this project is to develop a functional cardiac tissue construct with enhanced host integration capabilities as a novel strategy to replace damaged myocardium. We have developed a novel templated fibrin scaffold seeded with cells to promote functional integration. Fibrin is an ideal scaffold material because it can be autologous, improves cell attachment and growth, and degrades into natural byproducts that can induce angiogenesis. The novel scaffold architecture includes 1) microchannels spanning the length of the scaffold, allowing alignment and organization of cells to mimic native cardiac tissue structure, and 2) micropores to enhance construct survival by improving nutrient delivery and waste removal. The dense fibrin scaffolds (stiffness = 16.0 ± 3.0 kPa) had mechanical properties closer to native myocardium than fibrin gels (0.5 to < 7 kPa). Centrifuge seeding with a tri-cell mixture of cardiomyocytes, endothelial cells, and fibroblasts increased scaffold stiffness (38.3 ± 8.9 kPa) to values near neonatal myocardial tissue (~40 kPa). Stiffness decreased over time in culture (25.2 ± 3.1 kPa, Day 6), which may indicate ECM formation and scaffold degradation. Patches of beating cells were observed inside channels within two days in culture. After three days in culture, histology showed cardiomyocyte and fibroblast alignment and immature lumen formation. These results indicate micro-templated fibrin scaffolds are a unique and viable platform for cardiac tissue engineering. This work supported by NIH HL064387 (MR, MS, BR) and NSF GRFP (KT).

3736-Pos

Cardiac Specific Overexpression of N-RAP in Transgenic Mice

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The muscle specific protein NRAP plays a role in myofibril assembly and is upregulated in mouse models of dilated cardiomyopathy. We sought to determine if increased N-RAP expression would directly lead to a cardiomyopathy phenotype. Novel transgenic lines were developed using the tet-off system with transgenic N-RAP expression requiring the tetracycline transactivator (tTA). tTA was introduced by mating the N-RAP transgenic animals with well-characterized animals carrying the tTA transgene controlled by the cardiac specific alpha-myosin heavy chain promoter. Multiple founder lines were examined and lines showing the most significant increase in NRAP expression were used for further investigation. N-RAP expression in theses animals was up to 2.5 times greater than control littermates as determined by western blot analysis. Histological examination of hearts from ~12 week old transgenic mice