Proteasome Activity is Reduced at the end of Pregnancy and Fully Restored to Non-Pregnant Levels One Week Postpartum in the Murine Heart

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The proteasome is the major protein degradation system in the heart, and its activity has been shown to be affected during pathological cardiac diseases. Proteasome dysfunction in the hypertrophic heart leads to accumulation of abnormal proteins and has been proposed to contribute to the transition to heart failure. Pregnancy places an increased demand on the healthy female’s heart resulting in ventricular hypertrophy and diastolic dysfunction as a result of volume overload and increased stretch and force demand. Since the molecular signature of pregnancy-related heart hypertrophy differs significantly from that of pathological hypertrophy, we investigated if the proteasome proteolytic pathway is affected by pregnancy in the mouse heart. We measured the transcripts and protein levels of proteasome subunits as well as proteasome activity in four groups of female mouse hearts: i) non pregnant (NP) at diastolic stage, ii) late pregnant (LP), iii) one day post-partum (PP1) and iv) 7 days post-partum (PP7). Real Time PCR showed that the transcript levels of RPN2 and RPT4 (subunits of 19S) as well as PP7. Real Time PCR showed that the transcript levels of RPN2 and RPT4 (subunits of 19S), RPN2 and RPT4 (subunits of 20S) did not change with pregnancy. Western blot analysis of heart lysates also revealed no significant differences in the expression levels of β7 (a subunit of 20S), RPN2 and RPT4 (subunits of 19S) subunits in the four groups mentioned above. The β1 (caspase-like) and β2 (trypsin-like) activities of the proteasome were significantly decreased in LP. The β5 (chymotrypsin-like) activity was significantly decreased 1 day post-partum. Interestingly, all three proteolytic activities of the proteasome were restored to normal levels 7 days post-partum. These results suggest that the proteasome proteolytic pathway is affected by pregnancy and is restored to NP levels soon after delivery.

Sepsis Related C5a Peptide Causes Calcium Overload in Adult Cardiac Myocytes

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Septic cardiomyopathy is an acute cardiac syndrome that occurs after the onset of sepsis due to infectious agents such as bacteria, viruses and fungi. During septic cardiomyopathy cardiac output falls due to waning contractile function of the heart. However, very little is known about the precise cause of cardiac failure in cases of sepsis. One factor induced during sepsis is the complement activation product C5a. C5a is a peptide that acts through a G-protein coupled receptor (C5AR), and affects cardiac myocyte contractility by unknown mechanisms. Here we have tested the effect of C5a peptide on single adult cardiac myocyte calcium homeostasis. Cardiac myocytes were isolated from healthy rats and intracellular calcium transients were monitored (fluo-4AM) before and after C5a peptide treatment. Intracellular calcium was monitored by two different methods: 1) using a conventional photomultiplier tube and, 2) using a high speed digital CCD camera (200frames/s) to image whole cell calcium transients and waves. Recombinant C5a was applied to cardiac myocytes during electrical pacing (0.5Hz, 40V). After application of C5a (82ng/mL) intracellular calcium concentrations and calcium transient amplitudes initially rose (from F/F0 = 1.0 to F/F0 = 1.46 ± 0.2 vs. 1.32 ± 0.04, n=4) and prolonged after C5a addition (half width = 260.2 ± 29.0 ms to 318.7 ± 47.1 ms) and spontaneous calcium transients and waves were observed in the diastolic period between electrical stimuli. Consequently the amplitude of calcium transients and contractions varied from stimulated beat to beat after C5a addition. Paradoxically at higher pacing frequencies (3Hz) calcium transient amplitude was smaller after C5a application (F/F0 = 1.46 ± 0.2 vs. 1.32 ± 0.04, n=4) and prolonged (half width = 127.0 ± 1.15 vs. 167.0 ± 15.6 ms). Spontaneous calcium transients were also observed in the absence of electrical stimulation following C5a treatment. These data suggest that C5a peptide acts through its receptor C5AR to cause cardiac myocyte intracellular calcium overload.
Drosophila melanogaster possess a simple linear heart tube and constitute an excellent genetic model system with which to investigate the effects of cardiomyopathic mutations. 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). Ex-cised fly hearts (controls) 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 an 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 Ca2+ signaling. LPS causes Ca2+ signaling anomalies which include delayed afterdepolarizations (DADs) and Ca2+-enhanced early afterdepolarizations (EADs). We find that co-cultures with cells co-mingled can prevent the consequences of LPS on the cardiomyocytes. The sequences of LPS are alterations in the normal [Ca2+]t, 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 spares 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 MSC. 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 will 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 [Ca2+]t, (116±34 ms vs. 155±40 ms) and time to 50% [Ca2+]t, (352±87 ms vs. 296±49 ms). Furthermore, quantitative videomicroscopy of resulting single cell contractions suggests that cardiomyocytes from both sources exhibit faster rates of rise (1.7±1.4 ms vs. 1.6±0.4 ms), contraction amplitude (4.2±1.6% vs. 4.4±2.1%), time to peak contraction (0.346±0.135 sec vs. 0.339±0.214sec), maximum contraction velocity (6.34±5.05um/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 µg 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 µM -1sec-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 levels of 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 myofibrillar assembly and is up-regulated 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 these 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