structure is not critically modified, or the ER milieu significantly altered. In the present study, we found that skelCSQ trafficked differently from cardCSQ in nonmuscle cells and neonatal heart cells. In fact, the distribution of skelCSQ was unique among known ER markers. SkelCSQ concentrated in a membrane compartment that was juxtaposed and distal to ER containing the cardiac isoform. SkelCSQ was contained in novel streaming tubules and vesicles aligned on tufts of microtubules. Consistent with immunofluorescence microscopy were the structures of the two Asn316-linked glycans on CSQ isoforms, with skelCSQ glycans trimmed beyond the Man9,8 that are indicative of proximal ER. Despite the complete non-overlap of skelCSQ and cardCSQ compartments, the two proteins co-localized in early ER when co-overexpressed, suggesting heteropolymer formation. The present study indicates that skelCSQ, in contrast to cardCSQ, evades ER polymerization, and polymerizes in the next distal secretory compartment, an early subcompartment of ERGIC. We conclude that different subcellular localizations for skelCSQ and cardCSQ result from a difference in the lumenal requirements for polymerization of each of the two CSQ isoforms, leading to ER retention (cardCSQ) or retention in a contiguous intermediate compartment (skelCSQ).

2655-Pos Board B625

Calumenin Knock-down (kd) Enhances Ca^{2+} Cycling Ability In Hl-1 Cells

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Calumenin is a multiple EF-hand Ca^{2+}-binding protein localized in the sarcoplasmic reticulum (SR) lumen. Evidence of the interaction between calumenin and SERCA2 in rat cardiac SR was shown recently (*Mol. Cells*, 26:265-269, 2008). To elucidate the possible role of calumenin in cardiac excitation-contraction (E-C) coupling, calumenin was knocked down by transfection of mouse cardiac cell line (HL-1 cells) with calumenin specific siRNA oligonucleotides. After 72 hrs of transfection, calumenin protein level was reduced by 75% without any obvious changes in the expression levels of other E-C coupling proteins such as RyR2, SERCA2, NCX, CSQ and PLB. A field stimulation (1Hz) of KD cells (n = 58) led to significantly increased Ca^{2+} transient peak height (1.02 ± 0.02 *vs.* 0.82 ± 0.03 fura-2 ratio at 340 and 380 nm, $p < 0.05$), decreased time to peak (0.093 ± 0.001 *vs.* 0.107 ± 0.003 s, $p < 0.05$) and time to 50% baseline (0.172 ± 0.005 *vs.* 0.235 ± 0.006 s, $p < 0.05$) as compared to control cells (n = 44). On the other hand, the SR Ca^{2+} load remained unchanged in KD cells. Pull-down experiments with GST fusion proteins showed that calumenin interacts with both RyR2 and SERCA2 in a Ca^{2+} dependent manner. Taken together, the present results suggest that calumenin is related to SR Ca^{2+} homeostasis. Currently, the molecular interactions between calumenin and SERCA2 or RyR2 are being examined by using various deletion mutants.

2656-Pos Board B626

Interaction between Cardiac Ryanodine Receptor and FK506-Binding Protein Revealed by Cryo-EM and FRET

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Type 2 ryanodine receptor (RyR2) is the major calcium release channel in cardiac muscle. Abnormal calcium release through a dysfunctional RyR2 has been implicated in certain types of sudden cardiac death and heart failure. A 12.6kDa FK506 binding protein (FKBP12.6) tightly associates with RyR2, and stabilizes the close state of RyR2 calcium channel. One proposed mechanism that underlies RyR2 channel dysfunction is the destabilization of the RyR2-FKBP12.6 interaction. In the present study, we mapped the location of green fluorescent protein inserted after residue Tyr-846, near the amino-terminal diseases-causing mutation hotspot, in the three-dimensional (3D) structure of RyR2 by cryo-electron microscopy (cryo-EM). The location of the inserted GFP was found to be close to the previously mapped FKBP12.6 binding site. Based on the structural information that we have learned from 3D cryo-EM, we designed a fluorescence resonance energy transfer (FRET) pair by inserting a yellow fluorescent protein in RyR2 after residue Tyr-846, and attaching a cyan fluorescent protein to FKBP12.6. By monitoring the FRET signals between the donor and acceptor, we are investigating the interaction dynamics between RyR2 and FKBP12.6.
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Effect of Stem Cell Transplantation on the Calcium Signaling in Adult Ventricular Myocytes

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Bone marrow derived stem cells (MSCs) are often discussed as a potential source for cardiac replacement tissue. Transplantation of undifferentiated cells into cardiac infarct regions has been shown to decrease infarct size and preserve cardiac function but the impact the cells have through paracrine effects or intercellular coupling remains to be determined. To determine how MSCs influence the excitability of cardiac myocytes we established a co-culture between freshly isolated mouse ventricular myocytes and dissociated MSCs. After 3 hrs of co-culture the cells were loaded with the Ca^{2+} indicator Fluo-4/AM and the Ca^{2+} handling properties of ventricular myocytes were analyzed at a stimulation frequency of 0.5 Hz. In comparison to control myocytes (ctrl) cardiomyocytes that co-localized with MSCs (co-MSC) exhibited a significantly increased Ca^{2+}-transient amplitude (F/F_o: ctrl: 2.3 ± 0.5, n =8; co-MSC: 3.5 ± 1.2, n=4). In addition, the transient duration at 50% (APD_{50}: ctrl: 457 ± 61 ms to co-MSC: 360 ± 33 ms); and 90% inactivation (APD_{90}: ctrl: 1.31 ± 0.15 s; co-MSC: 1.08 ± 0.16 s) was significantly shortened. We have previously demonstrated that stem cell derived cardiomyocytes and adult myocytes can establish intercellular coupling within 1 hour of co-culture. However, in heterocellular pairs of ventricular myocytes and MSCs no change MSC $[Ca^{2+}]_i$ could be determined upon stimulation of the myocyte. The data indicate that MSCs modulate substantially the Ca^{2+} signaling properties of adult ventricular myocytes and therefore could have as substantial anti-arrhythmic effect upon transplantation. It remains to be determined if intercellular coupling is necessary to establish this effect.

2658-Pos Board B628

Skeletal Myotubes from Adult Mice in a Cardiac Environment

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The present study was designed to evaluate the functional impact of cardiac environment on the differentiation of skeletal myoblasts and Ca2+ signaling in electrically stimulated myotubes. Adult mice FDB muscle myoblasts were cultured alone or in co-culture with cardiomyocytes contractile monolayers. After 5 days of differentiation in a cardiac environment, the number of skeletal myogenic cells and myotubes were fourfold higher than control. Cardiac environment changed parameters of myotube calcium transients (Fluo-4AM) associated with Ca2+ removal mechanisms without affecting parameters related to Ca2+ release. The values (mean ± sem) of half width (HW) and single exponential decay time constant (τ), obtained from mono-cultured myotubes (MMy) were 209.42±34.72 ms and 415.16±55.12 ms (n=9), while for co-cultured myotubes (CMy) they were 554.94±67.38 ms and 1340.17±330.73 ms (n=9), respectively. HW and τ from neighboring cardiomyocytes (CM) were 274.78±50.17 ms and 387.72±47.98 ms (n=5). The transient rise time (RT) values for MMy and CMy were 33.46±8.79 (n=9) and 29.66±8.97 ms (n=9) while the amplitude values (ΔF/F) for the two cases were 0.94±0.09 (n=9) and 1.00±0.07 (n=9), respectively. In the absence of external Ca2+ (0.5mM EGTA) the parameters associated with Ca2+ removal were not affected, while those related to release mechanisms were as follows: RT = 105.38±24.93 ms (n=5) and 80.87±18.28 ms (n=5) for MMy and CMy respectively, and ΔF/F=0.56±0.11 (n=5) and 0.71±0.12 (n=5) for MMy and CMy, respectively. Thus, transient sensitivity to extracellular Ca2+ was not affected by coculture since both CMy and MMy were similarly modified by exposure to 0Ca2+. We conclude that at an early coculture stage, a cardiac environment facilitates skeletal muscle differentiation without affecting functional attributes characteristic of skeletal muscle, with the exception of a selective effect on Ca2+ removal parameters.

2659-Pos Board B629

Relationship of Ryanodine Receptors to the Sarcolemma in Rabbit Ventricular Myocytes

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To investigate the spatial relationship between the sarcolemma and ryanodine receptors (RyRs) we dual-labeled cells using Alexa fluor dyes and simultaneously imaged them with confocal microscopy. We deconvolved the images and subjected them to digital processing. We obtained three-dimensional reconstructions from cells in two configurations: lying flat and standing on end. In the flat configuration, RyR clusters appeared to be arranged in sheets near Z-disks. The distance between sheets was ~2 μm. Although some clusters are closely associated with detectable sarcolemma, the majority of them are not (>70%). With cells standing on end in agar we obtained XY scans orthogonal