arachidic) consistent with the changes observed in liver's metabolome. Higher liver AMP levels in HCR *vs.* LCR suggest activation of AMPK signaling, in agreement with the enhanced autophagy/mitophagy observed in HCR. Importantly, the higher relative LCR/HCR fold-changes of acetylated intermediates, in both liver and serum, agrees with the idea of widespread increased acetylation, compatible with the higher concentration of AcCoA measured in cardiomyocytes from LCR *vs.* HCR. Metabolomics reveal systemic, organ-concerted (liver-serum-heart), metabolic remodeling compatible with higher energetic performance, better preserved mitochondrial turnover, preferential lipid oxidation, and improved control of acetylation in HCR leading to extended longevity and better functional capacity.

1337-Pos

The Complex Crosstalk between Parvalbumin and Mitochondria Regulation through Changes in Mitochondrial Dynamics

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Parvalbumin (PV) is a cytosolic Ca^{2+}-binding protein, or better perceived as Ca^{2+} signal modulator, that together with mitochondria and ER compartments play an important role in Ca^{2+} signaling, buffering and sequestration. Moreover, antagonistic regulation of PV expression levels and mitochondrial volume occurs in several *in vivo* and *in vitro* model systems. The aim of this study was to examine mitochondrial dynamics (fusion, fission, mitophagy) in genetically modified MDCK cell lines characterized by PV overexpression or PV downregulation. Cell morphology was assessed by live-cell confocal imaging and 3D-reconstruction using Imaris software. Mitochondrial fusion was analyzed as fluorescence change of the photo-convertible protein mEOS2. Selective removal of damaged mitochondria by the process of autophagy (mitophagy) was quantified from TEM images, as well as from confocal images showing fluorescence of YFP-Parkin, GFP-LC3-C and mKeima proteins. Transcript and protein expression levels of selected genes were evaluated by RT-qPCR and Western blot, respectively. Volumetric analyses revealed the antagonistic regulation of PV and mitochondrial volume in MDCK cells. Control (PV-negative) cells showed typical epithelioid morphology with elongated mitochondria and frequent fusion-fission events. PV-overexpression resulted in smaller, roundish PV-MDCK cells and shorter mitochondria, the latter related to reduced fusion rates and decreased expression of genes involved in mitochondrial fusion. They also exhibited increased mitophagy, a likely cause for the decreased mitochondrial volumes and the smaller overall cell size. PV down-regulation by an shRNA approach in PV-MDCK cells reverted the mitochondrial morphology to the condition prevailing in parental MDCK cells, resulting from faster mitochondrial movement and augmented fusion rates. Hence, PV-modulated, bi-directional and reversible mitochondrial dynamics are key to regulation of mitochondrial volume.

1338-Pos

The Effects of Astaxanthin on Amyloid Beta Challenged Hippocampal Cell Growth and Mitochondrial Function During Hypoglycemia

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In the next 30 years, the amount of individuals diagnosed with Alzheimer's disease is expected to reach 30 million while the rate of diabetes mellitus is also projected to rise as well. In diabetes, hypoglycemia is a common consequence due to therapy. Previous research has shown a potential link between Alzheimer's disease and diabetes. This study sought to determine if Astaxanthin, ATX, could prevent mitochondrial dysfunction from the compounded effects of amyloid β (Aβ) plaque and hypoglycemia. Growth patterns, ATP production, and ROS generation were examined in 2 μM, 5 μM, 25 μM (hypoglycemic groups) and 2 mM or 5 mM glucose, and then treated with or without ATX or Aβ. When hypoglycemic groups were treated with ATX, their growth patterns were either comparable to or increased. ATX and Aβ monomers treated cells demonstrated increased growth patterns over hypoglycemic cells treated with Aβ monomers. Aβ alone treated groups overall had significantly less growth than controls ($p < 0.05$). Hypoglycemic groups produced overall low levels of ATP when ATP production was analyzed. Cells cultured with Aβ demonstrated low levels of average fluorescence generated by ROS production using the MitoSox assay while ATX groups actually produced higher to normal levels of ROS. Cells grown in the presence of Aβ and ATX generally produced more ROS than just Aβ groups. Thus, hypoglycemia does appear to compound the effects of Aβ monomers on hippocampal cells. ATX treatment demonstrated promise with increased cellular growth, which promotes usage of ATP by the cell and ROS production. This growth is also observed in the presence of Aβ and with other treatments may be useful in the treatment of these two disease states.

1339-Pos

Mitochondrial Membrane Potential Heterogeneity in Cancer Cells is Independent of the Cell Cycle and Influences Response to Hyperpolarizing Agents

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Mitochondrial membrane potential ($\Delta\Psi$) is sustained by metabolite flux through Voltage dependent anion channels (VDAC). Free α/β-tubulin heterodimers decrease VDAC conductance and high free tubulin in cancer cells decreases $\Delta\Psi$. The small molecule erastin antagonizes the inhibitory effect of tubulin on VDAC and increases $\Delta\Psi$. We found higher erastin-induced hyperpolarization in low $\Delta\Psi$ compared to high $\Delta\Psi$ cells. We **hypothesized** that heterogeneity of $\Delta\Psi$ is independent of cell cycle and determines the magnitude of drug-induced hyperpolarization. Our **AIM** was to quantitate $\Delta\Psi$ in cancer cells in relation with the cell cycle and after mitochondrial hyperpolarizing agents. **Methods**: Confocal fluorescence microscopy assessed $\Delta\Psi$ (TMRM, rhodamine 123) and plasma membrane potential (DiBAC4(3), PMPI). HepG2 and Huh7 human hepatocarcinoma cells and HCC4006 human lung adenocarcinoma cells were used to measure $\Delta\Psi$ in relative and absolute values. HepG2 were synchronized in G1 by serum starvation, early S by double thymidine block, and G2 by double thymidine block followed by nocodazole. **Results**: HepG2, Huh7, and HCC4006 cells displayed wide spectrum of $\Delta\Psi$ with median values of 20%, 25%, and 29% of maximal constitutive $\Delta\Psi$ respectively. HepG2 $\Delta\Psi$ range was −120 mV to −230 mV. $\Delta\Psi$ heterogeneity did not correlate with differences in plasma membrane potential. After synchronization in S, G1, and G2, $\Delta\Psi$ remained heterogeneous. The hyperpolarizing effect of erastin, paclitaxel (decreases free tubulin), and oligomycin (inhibits complex V) was higher in low $\Delta\Psi$ cells compared to high $\Delta\Psi$ cells (288% vs 148%, 334% vs 141%, and 137% vs 21% increase, respectively). **Conclusion**: Heterogeneity of $\Delta\Psi$ is independent of cell cycle in cancer cells. Constitutive $\Delta\Psi$ determines the magnitude of response to hyperpolarizing agents with different mechanisms of action, suggesting a cell specific modulation of $\Delta\Psi$.

1340-Pos

St. John's Worth Extract Induces Apoptosis and Inhibits Cancer-Related Inflamation in Basal Cell Carcinoma Cell Lines

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Basal Cell Carcinoma (BCC) is the most commonly diagnosed skin cancer in the world. It can be treated through standard surgical excision in most of the patients, but some patients can not be tolerate surgical procedures; therefore, it should be used non-surgical treatment for these kinds of patients. St. John's worth/Hypericum perforatum (HP) plant has biological active contents and is also used by local people in treatment of some diseases. The content of HP affects apoptosis. Apoptotic pathways are significant in cancer development and chemotherapeutic resistance. Furthermore, it is reported that anti-inflammatory agents are a potential target for cancer.

Thus, we studied if HP extract affects apoptotic pathways mediators; caspase-3, bax, bcl-2, gadd153, grp78, AIF and cell cycle G2/M checkpoint kinase; wee 1, and also inflammation mediators; iNOS, COX-2, cPLA2, NFkB expression in treated BCC cells.

Human basal cancer cell lines were obtained from ATCC, then were grown on Dulbecco's Modified Eagle's Medium (DMEM). Cell viability assay and cell homogenization were performed. One of the two BCC cell lines was given HP extract, whereas the other was not given. Then their activity of apoptotic pathways, cell cycle, inflammation mediators were analyzed by ELISA in these cultured cells. The data were analyzed statistically.

It was detected that HP extract treatment increased expression of caspase-3, bax, gadd153, grp78, AIF apoptotic proteins and cell cycle G2/M checkpoint

kinase- wee 1 although it decreased bcl-2 antiapoptotic protein and iNOS, COX-2, cPLA2, NFkB inflammatory mediators in BCC cells.
In this study, it is asserted that HP extract will be able to affect in the treatment of BCC due to characteristic feature of anti-inflammatory and wiping out cancer cells by affecting several intracellular pathways.

1341-Pos
Loss of Mgr2P Destabilizes the TIM23 Channel and Reduces Mitochondrial Emission of Reactive Oxygen Species
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The TIM23 complex is a hub for translocation of preproteins into or across the mitochondrial inner membrane. This dual sorting mechanism is currently being investigated, and in yeast it seems to be regulated by a recently discovered subunit, the Mgr2 protein. Deletion of Mgr2p has been found to delay protein translocation into the matrix and accumulation in the inner membrane. This result and other findings suggested that Mgr2p controls the lateral release of inner membrane proteins harboring a stop-transfer signal that follows an N-terminal acid presequence. However, the mechanism of lateral release is unknown. Also unknown is whether the presence or absence of yeast Mgr2p might modulate reactive oxygen species (ROS), like its mammalian counterpart, Romo1. Here, we used patch clamp electrophysiology and spectrofluorimetry to investigate the effect of Mgr2p deletion upon the channel activity of TIM23 and upon emission of ROS from mitochondria. Our results suggest that Mgr2p deletion both destabilizes the TIM23 channel and decreases ROS emission. However, these two effects seem independent from each other, because ROS emission was not affected in the presence of presequence peptides.

Posters: Emerging Techniques and Synthetic Biology

1342-Pos
Characterizing DNA Nanotube Networks Assembled via Y-Junction DNA Origami Seeds
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DNA nanotechnology offers a means to synthesize custom nano-structured materials from the ground up in a hierarchical fashion. While the assembly of DNA nanostructures from small (nanometer-scale) monomeric components has been studied extensively, a general model for the hierarchical assembly of rigid or semiflexible units into multimicron-scale structures remains elusive. To study such hierarchical assembly, we have developed a system for assembling extend networks of semiflexible DNA nanotubes. These nanotubes assemble from nanometer scale tiles into materials via the nucleated growth from sites on rigid, Y-shaped nanotube seeds. In this process, nanotubes first grow from these Y-shaped seeds to form 3-armed nanotube architectures. These architectures then in turn assemble into networks that include as many as 80 seeds and can extend over areas as large as 900 μm^2. We measure the kinetics of network growth and find that the assembly of these networks can be explained by a stochastic model of hierarchical assembly that assumes a single joining rate between DNA nanotube ends. Because the number of nucleation sites on the seeds and their spatial arrangement can be systematically varied by design, this system allows the assembly of a wide variety of networks and characterization of the assembly mechanisms that lead to different types of material architectures at length scales of tens to hundreds of microns. Further, by activating/deactivating the incorporated Y-shaped DNA origami junctions via strand displacement we are also able to direct networks to change form, suggesting a model system for understanding not only the formation of filament networks at this length-scale but also their reconfiguration.

1343-Pos
Combinational Genetically Encoded Toolbox for Cell-Surface Mucin Biopolymer Engineering
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Few approaches exist for the stable and controllable synthesis of bottlebrush biopolymer coatings on eukaryotic cells. Inspired by cellular mucins, we constructed a mix-and-match catalogue of cDNA bricks for biosynthesis of bottlebrush biopolymers of tunable size, glycan side-chain spacing, and charge density. Fusing the polymer cDNAs to additional bricks for leader tags, optical reporters, membrane anchors, and cytoplasmic motifs, we can generate a library of over 400 cDNAs, each encoding a polymer coating with unique chemical, physical, and optical properties. Notably, we found that we can tune the number of negatively charged sialic acids on our biopolymers through rational design of cytoplasmic motifs that impair or promote intracellular recycling. Applying this biology-by-parts approach, we have generated new anti-adhesive coatings on cells and tuned the diffusion and transport of transmembrane proteins on the cell surface. We have also found that clustering of serine or threonine contributes to the extension of O-glycans. Thus, we expect that our parts inventory can be broadly used to study in-vivo glycosylation, engineer the adhesive properties of cells in biotechnology, as well as the transport and functioning of native receptors on the cell surface.

1344-Pos
Creation of Scaffold-Free Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes (hiPSC-CMs) Cell Sheets for Drug Screening and Regenerative Medicine
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We describe a method to create a liquid surface to support self-assembly of cells, the curvature of which can be altered to produce cell sheets with different shapes. The assembly comprises a removable polydimethylsiloxane (PDMS) cell mold and a standard 96-well polystyrene (PS) microplate. The cell mold is inserted into each well of the plate to create an air-filled chamber between PDMS and PS. The sealed volume of air in the chamber provides mechanical support for the liquid inside the PDMS cell mold, allowing the bottom surface of the suspended liquid to be flat which we show to provide a supporting surface for self-assembly for cell cultures. Thereby, small scale scaffold-free cell sheets can be produced easily and repeatably. The size of the cell sheets can be varied from 1 to 3 mm depending on the size and shape of PDMS cell mold used. The thickness of the cell sheet ranges 0.06-0.1 mm day 1 after plating, representing 3-5 cell layers. The electrophysiology and contractility of the 3D culture layer containing commercially available human-induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) was investigated using the CellOPTIQ® platform (Clyde Biosciences Ltd). Visible scaffold-free hiPS-CMs cell sheets formed after 24 hours of the cell seeding showed spontaneous rhythmic electrical and contractile activity; by comparison, traditional 2D hiPS-CMs cell culture required several days before comparable activity was evident. The 3D culture layer formed with the present method is thicker and more uniform than the traditional 2D monolayer cell culture. Scaffold-free 3D cell sheets made with the method therefore constitute pure tissue-like cell structures which are suitable for high throughput drug screening and have potential also for regenerative medicine.

1345-Pos
Integration of Reaction-Diffusion Master Equation and Brownian Dynamics Methodologies to Simulate a Minimal Cell
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Simulating whole-cells at the genome scale involves the integration of models which operate at incompatible length, time, and concentration scales. Hybrid simulation schemes are necessary to bridge these scales. Progress is presented on a method combining the reaction-diffusion master equation (RDME) with Brownian dynamics (BD) into Lattice Microbes, a GPU-based RDME simulation code. RDME is used for protein diffusion, while BD is used for diffusion of particles larger than the subvolume size (ribosomes). The BD integration is performed on the GPU, allowing both simulations to progress in lockstep. Owing to the dense molecular crowding observed in living cells, accounting for excluded volume interactions is necessary to predict the spatial distribution and diffusion time scale of chemical species. Particles interact through a shared excluded volume field which influences the spatial transition rate of RDME