voltage-dependent anion channel isoform 2 (VDAC2). Here, we show that HCC induced by carcinogenic compounds, aflatoxin or DEN is highly sensitized to tBid-induced OMM permeabilization and cyto c release when compared to normal liver. Expression levels of VDAC2 and Bak were also higher in HCC than in control liver. The role of the VDAC2-Bak pathway in the differential tBid sensitivity was validated by overexpression of VDAC2 in primary hepatocytes. In HCC cells, elevation of Bak was also associated with an increased level of Mcl-1, an inhibitor of Bak. Furthermore, both constitutive and drug-induced genetic deletion of Mcl-1 in mouse embryonic fibroblasts caused sensitization to tBid-induced OMM permeabilization in the absence of a change in Bcl-xL or Bax . Based on these results, we are evaluating the possibility of selective killing of HCC cells by the combination of a Bak activator and an Mcl-1 inhibitor.

3368-Pos Board B523

Which Domain of VDAC2 is Necessary for Bak Insertion to the Outer Mitochondrial Membrane and tBid - Induced Cytochrome C Release?

Shamim Naghdi[1], Peter Varnai[2], Soumya Sinha Roy[1], Laszlo Hunyady[2], Gyorgy Hajnoczky[1].
[1]Thomas jefferson university, philadlephia, PA, USA, [2]Semmelweis University, Budapest, Hungary.

VDAC proteins represent a main component of the outer mitochondrial membrane (OMM). The VDAC family is composed of 3 isoforms with more than 70 percent similarity. Although their primary role was known to be in ion and metabolite transport between mitochondria and cytosol, it has been discovered that VDACs are also involved in apoptotic pathways. We have recently found that specifically the VDAC2 isoform is needed for tBid-induced cytochrome c release, due to its role in supporting Bak insertion to the OMM (Roy et al. 2009. EMBO Rep.10:1341-7).

To determine the domain(s) of VDAC2 which supports Bak insertion, VDAC1 and VDAC2 amino acid composition compared and the unique attributes of VDAC2 sequence were considered for mutational analysis. To test functional significance of the changes in VDAC2 or VDAC1, VDAC2-/- MEF cells that are resistant to tBid, were transfected with the mutants and insertion of Bak to the OMM and cytochrome c release were monitored in permeabilized cells by immunoblotting. Previously, we showed that the VDAC2-specific N terminal tail and cysteins are dispensable for tBid-induced OMM permeabilization. To approach the remaining differences four chimeras were prepared: VDAC1 (1-185)VDAC2(198-295), VDAC2(1-12)VDAC1(1-185)VDAC2(198-295), VDAC2(1-188)VDAC1(177-283) and VDAC2(13-188)VDAC1(177-283). These studies revealed that tBid dependent OMM permeabilization is only supported by VDAC2(1-188)VDAC1(177-283) and VDAC2(13-188)VDAC1(177-283), indicating that the first two third of VDAC2 is required and sufficient for Bak targeting to the OMM and tBid induced cytochrome c release.

3369-Pos Board B524

Outer Mitochondrial Membrane Protein Distribution and Function Depend on Mitochondrial Fusion

David Weaver[1], Xingguo Liu[1], Veronica Eisner[1], Peter Varnai[2], Laszlo Hunyady[2], Gyorgy Hajnoczky[1].
[1]Thomas Jefferson University, Philadelphia, PA, USA, [2]Semmelweis University, Budapest, Hungary.

Cells and tissues deficient in mitochondrial fusion have been shown to have defects linked to the exchange of inner membrane (IMM) and matrix components, particularly mitochondrial DNA. Outer membrane (OMM) constituents originate in the cytoplasm, thus the role of intermitochondrial transfer of OMM components by fusion remained unexplored. Here we show that fibroblasts lacking the GTPases responsible for OMM fusion, Mfn1/2, have a more heterogeneous distribution of OMM proteins than wild-type cells, and in particular that heterogeneity of pro-apoptotic Bak leads to dysregulation of Bid-dependent apoptotic signaling. Homogeneous distribution of Bak is partially rescued by introduction of Mfn2 into Mfn1/2-/- cells. Furthermore, fusions between mitochondria lacking and containing Bak result in hybrids sensitive to Bid. Proteins with different modes of OMM association display varying degrees of heterogeneity in Mfn1/2 /- cells and different kinetics of transfer during fusion in fusion-competent cells. Efficient coupling of OMM to IMM fusion depends on the presence of both Mfn isoforms and is antagonized by the mitochondrial fission protein, Drp1. Thus, OMM function depends on mitochondrial fusion and is a locus of dysfunction in conditions of fusion deficiency.

3370-Pos Board B525

Mitochondrial Fusion Dynamics in Skeletal Muscle of Healthy and Diseased Rat

Veronica Eisner[1], Guy Lenaers[2], Gyorgy Hajnóczky[1].
[1]Thomas Jefferson University, Philadelphia, PA, USA, [2]INSERM U-583, Institut des Neurosciences de Montpellier, Montpellier, France.

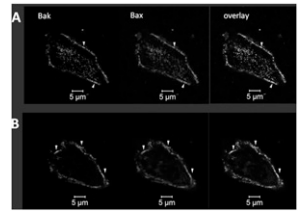
Mitochondria are highly mobile and dynamic organelles in many cell types. However, in the muscle, mitochondria are crammed into the narrow space among the myofilaments. Until now, it remained unclear if mitochondrial fusion occurs in and supports the contractile activity of skeletal muscle (SM). Here we applied mitochondria matrix-targeted DsRed and photoactivable GFP to study mitochondrial fusion in rat FDB SM fibers. In vivo electroporated fibers were imaged by confocal microscopy. When we tagged the mitochondria in ~5% of cell area with twophoton photoactivation of GFP, rapid spreading of fluorescence showed subsets of interconnected mitochondria, mostly in longitudinal direction. Matrix fusion occurred with a rate of 0.5 and 6.4 events/min in adult fibers and skeletal myotubes, respectively. Expression of Autosomal Dominant Optical Atrophy-causing mutants of the fusion protein Opa1 or ethanol exposure caused suppression of fusion, which is followed by mitochondrial dysfunction and late onset myopathies. When challenged by repetitive tetanic stimulation, ethanol exposed cells displayed intracellular Ca^{2+} dysregulation, appearing as a fatigue pattern. Ethanol also induced a decrease in Mfn1 protein levels, without significantly altering Mfn2, Opa1 or deltapsi. Depletion of Mfn1 alone was sufficient to suppress mitochondrial fusion in FDB fibers. Fusion inhibition was apparent before any cell dysfunction, suggesting that suppression of fusion is not secondary to other problems in the cells. To directly test the role of Mfn1 in Ca^{2+} regulation, RyR1-transfected control and Mfn1KO MEFs were stimulated with caffeine. Mfn1KO cells showed oscillatory Ca^{2+} transients that decayed faster than that in the control. Thus, fusion dynamically connects skeletal muscle mitochondria and serves as a target mechanism of both mutations and environmental cues to cause impaired excitation-contraction coupling.

3371-Pos Board B526

BCL-2 Proapoptotic Proteins Distribution in U-87 MG Glioma Cells before and after Hypericin Photodynamic Action

Katarina Stroffekova, Lucia Balogová, Mária Maslaňáková, Lenka Dzurová, Pavol Miškovský Miškovský.
Safarik University, Kosice, Slovakia.

Apoptosis is a key process in the development and maintenance of tissue homeostasis. This process of controlled cell death is tightly regulated by a balance between cell survival and damage signals. We focused our attention towards the intrinsic mitochondrial apoptotic pathway, where Bcl-2 family of proteins plays the major role. We were particularly interested in two pro-apoptotic players Bak and Bax. Here we investigated their role in apoptosis triggered by photodynamic action. We show the localization of Bax and Bak in U-87 MG human glioma cells incubated with photosensitizer hypericin (Hyp) before and after photodynamic action. Apoptotic stimulus by Hyp photodynamic action caused Bax translocation into mitochondria. However our results suggest that under these conditions there are two populations of mitochondria: one which contains Bax and Bak simultaneously, and is almost exclusively localized near the plasma membrane; the other which contains Bax only and is distributed throughout the cell. The different protein content and spatial distribution of these two populations suggest that they can play different roles in response to apoptotic stimuli.



3372-Pos Board B527

Stimulation of Bax Mitochondrial Localization by Bcl-xL

Thibaud Renault[1], Oscar Teijido[2], Gisele Velours[1], Yogesh Tengarai Ganesan[2], Florent Missire[1], Nadine Camougrand[1], Bruno Antonsson[3], **Laurent Dejean**[4], Stephen Manon[1].
[1]IBGC-CNRS, Bordeaux, France, [2]NYU College of Dentistry, New York, NY, USA, [3]Merck-Serono SA, Geneva Research Center, Geneva, Switzerland, [4]California State University of Fresno, Fresno, CA, USA.

Cytochrome c release, the commitment step of apoptosis, is regulated at the mitochondria through protein-protein interactions between the Bcl-2 family proteins. An imbalance of this interaction network due to the up-regulation of the proto-oncogenes Bcl-2 and/or Bcl-xL lead to a resistance to apoptosis and is associated with tumor formation. Bcl-xL overexpression act at the level of the mitochondrial outer membrane (MOM) by inhibiting Bax-mediated apoptosis; more particularly MAC formation and cytochrome c release. However, the molecular mechanisms through which Bcl-xL affect earlier steps of Bax-mediated apoptosis are not fully understood. Surprisingly, we found that mitochondrial Bax redistribution and change of conformation were not inhibited but rather spontaneously increased in response of Bcl-xL overexpression. In order to further investigate the molecular mechanisms involved in this

effect, different Bax and Bcl-xL mutants were co-expressed in yeast. We found that wild-type Bcl-xL increased the mitochondrial localization of Bax; but that this effect was lost when Bcl-xL mutants unable to physically interact with Bax were expressed instead of wild-type Bcl-xL. Also, Bax retrotranslocation from mitochondria to cytosol was also increased; suggesting that both translocation and retrotranslocation events were differentially stimulated by the expression of wild-type Bcl-xL. Further, a C-terminally truncated mutant of Bcl-xL, that lost its capacity to relocate to mitochondria, still increased the mitochondrial localization of and cytochrome c release activity of wild-type Bax. Finally, wild-type Bcl-xL protected activated Bax against proteolytic degradation. These data suggest that Bcl-xL, by acting as a modulator of mitochondrial Bax localization, may play an active role along the pathway leading to Bax activation.

3373-Pos Board B528

Mitochondrial DNA Nucleoid Redistribution after Mitochondrial Network Fragmentation as Visualized by 3D Super-Resolution Biplane Fpalm Microscopy

Andrea Dlaskova[1], Tomas Spacek[1], Jan Tauber[1], Lukas Alan[1], Jitka Santorova[1], Katarina Smolkova[1], Jaroslav Zelenka[1], Zdenek Svindrych[1], Joerg Bewersdorf[2], **Petr Jezek**[1].
[1]Institute of Physiology, Prague, Czech Republic, [2]Department of Cell Biology, Yale University, New Haven, CT, USA.

Mitochondrial (mt) network undergoes locally frequent fragmentation and fusion events. When integrated over time, its basic morphology encompasses highly interconnected mt reticulum, a single mitochondrion within the cell (1). Upon certain insults and/or pathological states fragmented network persists. MtDNA is organized in nucleoids containing assessor proteins and recruited proteins of mt replication/ transcription machinery. It is debated on their uniform size and whether a single nucleoid contains a single mtDNA molecule or up to average 6 mtDNA molecules. To image nucleoid distribution within mt network, we employed 3D super-resolution fluorescent photactivable localization microscopy of Biplane schema (1). Mt network of hepatocellular carcinoma HepG2 cells was imaged first by its matrix space using mtEos2 or as outer mitochondrial membrane contour using Eos2-conjugates of truncated FIS1 protein (not inducing massive fission, Eos2-FIS1tr). Resulting 3D images confirm the existence of highly-connected mt network and unlike conventional confocal microscopy, 3D BiplaneFPALM distinguished a hollow character of mt reticulum tubules when visualized by Eos2-FIS1tr. Upon network fragmentation, hollow max ~2 micrometer spheres occurred. Imaging of mt nucleoids confirmed the existence of ~1000 nucleoids per cell with size distribution from 50 nm to 300 nm. Optimized dual transfection strategy had to be employed for simultaneous imaging of network (mtEos or Eos2-FIS1tr) and nucleoids (mtSSB-PSCFP2). Images revealed an equidistant nucleoid distribution of an average distance of ~1 micrometer between nucleoids. Fragmentation by different agents led to observations of clusters of mt nucleoids within the spherical fragmented objects thus formed.

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3374-Pos Board B529

Integrating Mitochondrial Energetics, Redox and Ros Metabolic Networks: A Two-Compartment Model

Jackelyn M. Kembro, Miguel A. Aon, Raimond L. Winslow, Brian O'Rourke, **Sonia Cortassa**.
Johns Hopkins University, Baltimore, MD, USA.

To understand the mechanisms involved in the control and regulation of mitochondrial reactive oxygen species (ROS) levels, a two-compartment computational Mitochondrial Energetic-Redox (ME-R) model accounting for energetic, redox and ROS metabolisms is presented. The ME-R model incorporates four main redox couples ($NADH/NAD^+$, $NADPH/NADP^+$, GSH/GSSG, $Trx(SH)_2$/TrxSS). Main scavenging systems - glutathione, thioredoxin, superoxide dismutase, catalase - are distributed in mitochondrial matrix and extra-matrix compartments, and transport between compartments of ROS species (superoxide: $O_2^{\cdot-}$, hydrogen peroxide: H_2O_2), and GSH is also taken into account. Model simulations are compared with experimental data obtained from isolated heart mitochondria. The ME-R model is able to simulate: *i)* the shape and order of magnitude of H_2O_2 emission and dose-response kinetics observed after treatment with inhibitors of the GSH or Trx scavenging systems; and *ii)* steady and transient behavior of $\Delta\Psi_m$ and NADH after single or repetitive pulses of substrate- or uncoupler-elicited energetic-redox transitions. The dynamics of the redox environment in both compartments is analyzed with the model following substrate addition. The ME-R model represents a useful computational tool for exploring ROS dynamics, the role of compartmentation in the redox environment modulation, and the role of redox regulation in the control of mitochondrial function.

3375-Pos Board B530

DJ1 regulates Neuronal Mitochondrial Bioenergetic Efficiency

Elizabeth A. Jonas, Emma Lazrove, Panah Nabili, Kambiz N. Alavian.
Yale University, New Haven, CT, USA.

The progressive loss of midbrain dopaminergic neurons is the hallmark of Parkinson's disease (PD). Defects in mitochondrial electron transport and mitochondrial DNA replication predispose to the onset of PD. The protein products of several PD genes, including Parkin, Pink1 and DJ1 are known to localize to mitochondria; pathological mutations in these genes may disrupt mitochondrial function. In a previous study we found that the anti-apoptotic protein Bcl-xL enhances the efficiency of neuronal energy metabolism by increasing total cellular ATP levels while decreasing cellular oxygen use. Bcl-xL produces this effect in part through a direct interaction with the beta subunit of the F1Fo ATP synthase. The interaction causes a decrease in leak of H+ ions across the mitochondrial inner membrane, correlated with an increase in coupling of oxidative phosphorylation. We now show that the Parkinson's disease gene-encoded protein, DJ1 (PARK7), is also associated with the ATP synthase complex and has a similar regulatory effect on enzymatic activity of the synthase and on the coupling of oxidation to phosphorylation. Pathological mutations of DJ1 may disrupt mitochondrial efficiency leading to neurodegeneration of mesencephalic dopaminergic neurons. The exact site of the leak inhibited by Bcl-xL and DJ1 is now being determined, but likely resides in or adjacent to the c-subunit ring of the ATP synthase Fo. Improved mitochondrial metabolic efficiency that accompanies decreases in H^+ leak may result in long lasting changes in synaptic efficacy and survival in both healthy and at-risk neurons, suggesting a role for leak regulation in future therapeutic interventions.

3376-Pos Board B531

Identification of Mitochondrial Proteins regulated during Activation of GPER1-Leading to Cardioprotection

Jean Chrisostome Bopassa[1], Rong Lu[1], Harpreet Singh[1], Bjorn Olde[2], L.M. Fredrik Leeb-Lundberg[2], Ligia Toro[1], Enrico Stefani[1].
[1]University of California Los Angeles, LA, CA, USA, [2]Lund University, Lund, Sweden.

Several studies have demonstrated that G-protein coupled estrogen receptor (GPER1) can directly bind to estrogen and mediate its action. We previously demonstrated that GPER1 activation with G1, a specific agonist is cardioprotective in wild-type (WT) mice subjected to ischemia/reperfusion injury. Here, we identified regulated mitochondrial proteins associated with GPER1 activation by estrogen treatment in WT and GPER1-/- mouse hearts after ischemia/reperfusion. Isolated hearts from male WT (C57BL/6NCrL) or GPER1-/- mice were perfused using the Langendorff technique with Krebs Henseleit buffer, with and without (control) the addition of estrogen (40 nM). Hearts were subjected to 18 min global ischemia followed by 10min reperfusion. Mitochondria were isolated, and 2D-DIGE followed by mass spectrometry was performed. Proteins of interest were the ones (up- or down- regulated) in WT+E2 vs. WT-control that remained unchanged in GPER-/-+estrogen vs. GPER-/–control and WT-control vs. GPER-/–control. Robust changes of proteins were observed in 45 spots, out of which 14 were down regulated and 31 up regulated. In these 45 spots, 52 unique proteins were identified. Among the proteins identified, estrogen treatment induced the regulation of enzymes mostly involved in electron transfer chain and ATP production. Estrogen action involved the down-regulation of filament proteins (filamin A,B,C), the up-regulation of proteins that activate transcription, and proteins involved in the contractile system (tropomyosin and myosin). Further, estrogen treatment is associated with regulation of proteins acting in stress (stress-70 protein, and 60 kDa heat shock protein), in cell communication (glial fibrillary acidic protein), and in signaling pathways (membrane-associated phosphatidylinositol transfer protein). Finally, estrogen down-regulated the mitochondrial inner membrane protein, this protein controls mitochondrial cristae morphology. In conclusion, rapid estrogen effects through GPER1 activation are associated with the down- or up-regulation of mitochondrial and non-mitochondrial proteins likely in close contact to mitochondria.

3377-Pos Board B532

Adrenergic Stimulation Accelerates Mitochondrial Ca^{2+} uptake by PYK2-Dependent Phosphorylation of Mitochondrial Ca^{2+} Uniporter in Cardiac H9C2 Cells

Jin O-Uchi, Bong Sook Jhun, Shey-Shing Sheu.
Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA, USA.

Background: Recent break-through discovery in the molecular identity of mitochondrial Ca^{2+} uniporter (MCU) opens the new possibilities for applying