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?

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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

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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

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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

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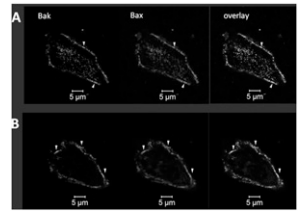
Apoptosis is a key process in the development and maintenance of tissue homeostasis. This process of controlled cell death is tightly regulated by a balance between cell survival and damage signals. We focused our attention towards the intrinsic mitochondrial apoptotic pathway, where Bcl-2 family of 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

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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