15 Å to 50 Å upon pore-formation. On the other hand, the distance between residues near the top of the hairpin, i.e., 75R1 and 122R1, which are located on αH2 and at the N-terminus of αH5, respectively, changes from approximately 20 Å to 25 Å. These results suggest that upon pore formation the layers covering the αH5-αH6 helical hairpin structure in BAK open up, exposing the helical hairpin structure for membrane insertion. These results are consistent with the aforementioned hypothesis regarding the conformational changes associated with the pore-forming Bcl2 proteins upon membrane permeabilization.

2404-Pos

Amphipathic Tail-Anchoring Peptide and BH3 Peptide Induced Mitochondrial Permeabilization and Apoptosis are Mechanistically Distinct

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Pro-apoptotic Bcl-2 homology domain-3 (BH3) peptides and mimetics have been developed as cancer therapeutics. Unfortunately, their cytotoxic effects are reduced in certain cancer cells by altered expression levels of various Bcl-2 family proteins. We recently found that the amphipathic tail-anchoring peptide (ATAP) from Bfl-1, a bifunctional Bcl-2 family member, displayed strong pro-apoptotic activity by permeabilizing the mitochondrial outer membrane. In this study, we tested if the activity of ATAP requires other cellular factors and whether ATAP has an advantage over the BH3 peptides or mimetics in targeting cancer cells. We reconstituted the membrane permeabilizing activity of ATAP in liposomes and found that ATAP rapidly released fluorescent molecules of the size of cytochrome c, suggesting that ATAP membrane permeabilizing activity is independent of other protein factors. ATAP permeabilized the membrane with more efficiency and potency than tBid-activated Bax protein, and unlike Bax whose pro-apoptotic activity was significantly blocked by Bcl-2, the activity of ATAP in both liposomes and cultured cells were only marginally inhibited by Bcl-2. While the pro-apoptotic activity of BH3 peptides was largely inhibited by either overexpression of Bcl-2 or Bcl-xL or nullification of Bax and Bak in cells, the apoptotic function of ATAP was not affected by these cellular factors.
Since ATAP can specifically target to mitochondria membrane and its potent apoptotic activity is not dependent on the content of Bcl-2 family proteins, it represents a promising lead for a new class of anti-cancer drugs that can potentially overcome the intrinsic apoptosis-resistant nature of cancer cells.

2405-Pos

BCL-xL Regulates ATP Synthase and Synaptic Efficiency

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Anti-apoptotic BCL-2 family proteins such as BCL-xL play a crucial role in protecting cells from death. High levels of expression of BCL-xL are also key to the maintenance of life of certain cancer cells. Healthy adult neurons also contain high levels of BCL-xL, suggesting that BCL-xL plays a role in daily neuronal function. We have found previously that over-expression of BCL-xL in cultured neurons causes an increase in the number and size of synapses and an increase in synaptic activity, providing evidence that BCL-xL causes long term changes in synaptic efficacy and structure. We now describe that in cultured hippocampal neurons, BCL-xL overexpression enhances the availability of total cellular ATP by increasing the ATP/ADP ratio. BCL-xL specifically enhances mitochondrial ATP production even while producing a marked decrease in cellular oxygen use. Although BCL-xL is usually thought to function in the mitochondrial outer membrane, our findings suggest that it creates an increase in the efficiency of cellular energy metabolism by direct protein-protein interaction with the ATP synthase beta subunit at the inner membrane. We find that recombinant BCL-xL protein increases native brain ATP synthase enzymatic activity and that pharmacological inhibitors of BCL-xL decrease the enzymatic activity of the synthase complex. In patch clamp recordings of the isolated synthasomes, ATP seals a membrane ion leak that could decrease synthase efficiency. In contrast, BCL-xL inhibitors increase the leak. The leak is different from the oligomycin-sensitive H+ ion pathway, and is not sensitive to the membrane permeant ANT inhibitor, bongkrekic acid, or to inhibitors of MitoK_{ATP}. Our findings suggest that BCL-xL improves the efficiency of mitochondrial metabolism by helping to seal a leak in the ATP synthase complex. This may allow for increased synthesis of synaptic components during long term increases in synaptic activity.

2406-Pos

Effect of Different Lipid Compositions on Mitochondrial Outer Membrane Permeabilization Assisted by the Pro-Apoptotic Proteins tBID and BAX

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Apoptosis or programmed cell death is a conserved process that serves to remove excess, damaged or infected cells in all multi-cellular organisms. Dysregulation in apoptosis can elicit important pathological conditions such as cancer and degenerative diseases. Bcl-2 family proteins critically regulate most pathways of apoptosis at the level of mitochondria. In addition to the protein-protein interactions among the Bcl-2 family members, the interaction of Bcl-2 family members with the mitochondrial outer membrane (MOM) are also very important for the execution of apoptosis. Considerable evidence supports that the composition of OMM mediates the translocation of the pro-apoptotic activator tBID to the OMM, and the subsequent activation of the pore-forming protein Bax at MOM to induce apoptosis. We have carried out a systematic study on the effect of different lipids, such as cardiolipin, monolysocardiolipin, cholesterol, and ceramide, using an *in vitro* system of liposomes to study MOM permeabilization.

2407-Pos

Structure and Dynamics of an Apoptotic Model Membrane

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In order to address the dynamic structural changes of cell membranes during apoptosis, we have studied the effect of enzymatically generated ceramide (Cer), in equimolar mixtures of palmitoyl-oleoyl-phosphatidylcholine and egg sphingomyelin (SM). Hydrolysis of SM to Cer was achieved using the well characterized neutral sphingomyelinase from bacillus cereus. By combining high performance thin layer chromatography, synchrotron time-resolved small- and wide-angle x-ray-scattering and photon correlation spectroscopy we were able to correlate the compositional changes of the bilayers to membrane structural adaptations and modifications on the macroscopic level. We found that the hyperbolic increase of Cer levels leads to an instantaneous generation of a gel phase domain. The gel phase forms initially only in the outer membrane leaflet and explains the membrane budding observed previously (1). After about 150 min a constant Cer level of 32 mol % was reached. The membranes, however, continued to swell indicating structural rearrangements due to diffusion processes, vesicle rupture/fusion, or enzyme enclosure. We observe a monotonic growth of vesicle size initiating at about the same time in agreement with vesicle aggregation, reported previously (2). This effect can be understood qualitatively in terms of reduced membrane undulations of the gel phase bilayers. Hence, we present for the first time a structural time-line that bridges the molecular to macroscopic changes occurring during apoptosis. The biological relevance of our results are supported by a remarkable agreement with the kinetics observed in Jurkat cells (3).

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2408-Pos

The Effect of Ceramide on Model Membranes and Apoptotic Cells Determined by X-Ray Scattering, Solid State NMR, and Flow Cytometry

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Ceramides (Cer) are sphingolipids involved in the development of lung alveolar cell apoptosis (programmed death) and possibly in the clearance of apoptotic cells by alveolar macrophages. Typically, the clearance process is initiated by the binding of the phosphatidylserine (PS) receptor on the macrophage plasma membrane to PS which is externalized on the plasma membrane of the apoptotic (target) cell. We use a combination of molecular and cellular methods to determine the effect of ceramides on the ability of alveolar macrophages to engulf apoptotic cells. Engulfment experiments of labeled apoptotic Jurkat cells were performed with rat alveolar macrophages (AM) obtained via bronchoalveolar lavage. AM were treated with various ceramide species and efferocytosis was quantified by flow cytometry. Using small-angle X-ray scattering and solid state 2H NMR we determined how ceramides (C6:0, C18:1) affect the molecular organization and the physical properties of PS-containing membranes. By investigating model membranes with various Cer:PS:PC ratios and deuterated species we show how ceramides alter membrane thickness, bending rigidity, and the ordering of the lipid acyl chains. These studies can

lead to a better understanding of the molecular mechanisms responsible for apoptotic cell clearance. If the clearance process is impaired, apoptotic cells may progress to secondary necrosis, resulting in release of harmful cellular contents and tissue inflammation. (IUPUI Membrane Biosciences Signature Center grant.)

2409-Pos

Lipid-Induced Up-Regulation of Acyl-CoA Synthetase 5 Promotes Apoptosis in Human Hepatoctes

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Long chain acyl-CoA synthetases (ACSL) activate fatty acids for utilization by numerous metabolic pathways. Of the five mammalian ACSL isozymes known, ACSL5 is the only one located on mitochondria and thought to be involved in apoptosis. Fatty acids up-regulate ACSL5 and increase apoptosis susceptibility in human hepatocytes, thus, we hypothesize that ACLS5 is a promoting factor in hepatocellular lipoapoptosis. To investigate this mechanism, we have used immunochemical techniques and RNA interference as well as liquid chromatography, tandem mass spectrometry (LC-MS/MS). Fatty acid uptake led to up-regulation of ACSL5 expression and enzymatic activity in primary hepatocytes, HepG2 cells and steatotic liver. Over-expression of ACSL5 decreased HepG2 cell viability and increased susceptibility to TRAIL and TNFα, whereas knock down of ACSL5 reduced apoptosis susceptibility in fatty-acid treated HepG2 cells. Apoptosis sensitisation was accompanied by enhanced caspase-3/7 activity, but was not associated with up-regulation of DR4, DR5 or TNF-R1. By applying lipidomic techniques, we determined the effect of ACSL5 on the cellular amounts and subspecies of fatty acyl-CoAs as well as on sphingolipids, the downstream metabolites that are known to be important regulators of cell death and survival. High ACSL5 activity in HepG2 cells increased synthesis of long-chain acyl-CoAs by 50%, and enhanced ceramide and sphingomyelin levels by 2 to 3 fold. These results indicate that steatosis-induced up-regulation of ACSL5 increased apoptosis susceptibility in human hepatocytes and that alterations in sphingolipid metabolism might contribute to ACSL5-mediated apoptotic effects. We propose that ACSL5 could play a role in promoting fatty acid-induced lipoapoptosis in hepatocytes as an important mechanism in fatty liver-related disorders.

2410-Pos

Differential Susceptibility of Normal and Transformed Human Leukocytes to Hydrolytic Attack by Secretory Phospholipase A_2

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Previous experiments with cultured lymphoma cells demonstrated that secretory phospholipase A_2 ($sPLA_2$) distinguishes healthy cells from those that are dying by apoptosis or necrosis. This distinction depends on cell membrane properties including the amount of negative charge at the bilayer surface and the strength of interactions among neighboring phospholipids. These results raised two important questions. 1) Does the enzyme's ability to distinguish healthy and dying cells apply to normal human leukocytes? 2) Does $sPLA_2$ differentiate between normal and tumor cells? These questions were addressed by comparing membrane properties and susceptibility to hydrolysis among cultured transformed leukocytes and freshly-isolated human neutrophils and lymphocytes. Membrane properties were assessed by flow cytometry, merocyanine 540 fluorescence spectra, trimethylammonium diphenylhexatriene fluorescence anisotropy, and two-photon scanning microscopy with laurdan. Similar to the behavior of transformed cells, normal human leukocytes resisted hydrolysis by $sPLA_2$. Upon addition of a calcium ionophore, ionomycin, the cells became vulnerable to hydrolysis, again analogous to the results observed with tumor cells. However, several important quantitative distinctions were observed. First, the various types of normal leukocytes responded differently to the enzyme; lymphocytes exhibited significantly greater rates of hydrolysis by $sPLA_2$ compared to granulocytes. Second, hydrolysis was substantially slower in normal cells compared to transformed cells. Third, the time required for ionomycin to induce cells to be attacked by $sPLA_2$ was greater in normal compared to transformed cells. Likewise, changes in membrane physical properties following ionomycin treatment were more subtle in normal cells than they were in transformed cells. These results suggest the possibility that $sPLA_2$ could function as a therapeutic ally during cancer chemotherapy to assist with the demise of tumor cells.

2411-Pos

Kinetic Evaluation of Cell Membrane Hydrolysis during Apoptosis by Human Isoforms of Secretory Phospholipase A_2

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Some isoforms of secretory phospholipase A_2 ($sPLA_2$) distinguish between healthy and damaged or apoptotic cells. This distinction reflects differences in membrane physical properties. Since various $sPLA_2$ isoforms respond differently to properties of artificial membranes such as surface charge, they should also behave differently as these properties evolve during a dynamic physiological process such as apoptosis. To test this idea, S49 lymphoma cell death was induced by glucocorticoid (6-48 h), thapsigargin (3-4 h) or calcium ionophore. Rates of membrane hydrolysis catalyzed by various concentrations of snake venom and human groups IIa, V, and X $sPLA_2$ were compared after each treatment condition. The data were analyzed using a model that evaluates the adsorption of enzyme to the membrane surface and subsequent binding of substrate to the active site. Results were compared temporally to changes in membrane biophysics and composition. Under control conditions, membrane hydrolysis was confined to the few unhealthy cells present in each sample. Increased hydrolysis during apoptosis and necrosis appeared to reflect substrate access to adsorbed enzyme for the snake venom and group X isoforms corresponding to weakened lipid-lipid interactions in the membrane. In contrast, apoptosis promoted initial adsorption of human groups V and IIa concurrent with phosphatidylserine exposure on the membrane surface. However, this observation was inadequate to explain the behavior of the groups V and IIa enzymes toward necrotic cells where hydrolysis was reduced or absent. The response to endoplasmic reticulum stress (thapsigargin) was intermediate between that observed for glucocorticoid and ionomycin. Thus, a combination of changes in cell membrane properties during apoptosis and necrosis capacitates the cell for hydrolysis differently by each isoform.

2412-Pos

VDAC1 Cysteine Residues: Topology and Function in Channel Activity and Apoptosis

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VDAC is proposed to control metabolic cross-talk between mitochondria and the cytosol, as well as apoptotic cell death. It has been suggested that apoptosis is modulated by the oxidation state of VDAC. Since cysteine residues are the major target for oxidation/reduction, we verified whether one or both VDAC1 cysteine residues are involved in VDAC1-mediated transport or apoptosis activities. To assess the function of the VDAC1 cysteines in channel activity and to probe cysteine topology, with respect to facing the pore or the bilayer, we used thiol-modifying agents; membrane permeable N-ethyl-maleimide (NEM); bulky, charged 5-fluorescein-maleimide (5-FM) and cross-linking reagent bis[maleimido]-ethane (BMOE). Bilayer-reconstituted VDAC conductance was decreased by 5-FM but not by NEM, while 5-FM had no effect on NEM-labeled VDAC conductance. BMOE formed dimeric VDAC1, suggesting that a VDAC1 cysteine residues is exposed and available for cross-linking. The results suggest that one of the VDAC1 cysteine residues faces the VDAC pore while the second is oriented toward the lipid bilayer. The positions of VDAC1 Cys127 and Cys232 with respect to the membrane and channel pore, were considered in light of proposed VDAC1 topology models. Mutated VDAC1 in which Cys127 and Cys232 were replaced by alanines showed channel activity as of native VDAC1 and when expressed in cells was localized to mitochondria. As with over-expression of native rVDAC1, cysteine-less rVDAC1 induced apoptotic cell death and underwent oligomerization upon apoptosis induction. The results suggest that the two cysteine residues are not required for VDAC1 oligomerization or apoptosis, as induced by H_2O_2, As_2O_3 or selenite, ROS producing agents.

2413-Pos

A Voltage Dependent Na+ Channel is Activated during Apoptosis in Xenopus Oocytes

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Apoptosis is regulated by a cascade of intracellular biochemical reactions. However, plasmamembrane bound ion channels are also essential for the apoptotic process. In previous studies we and other have found that K, Cl and Na channels of different types are upregulated early in the apoptotic process. Furthermore, block of these channels prevent or delay the apoptosis, suggesting a critical role of the channels in the apoototic process. In the present investigation we examined whether ion channels are upregulated in oocytes from the