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