Posters

Apoptosis

201-Pos Board B1
Mapping the VDAC1 Oligomerization Contact Sites and N-Terminus Translocation
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Accumulating evidence implicates the voltage-dependent anion channel (VDAC) as functioning in mitochondria-mediated apoptosis involving cytochrome *c* release, leading to caspases activation and apoptosis. The mechanisms regulating cytochrome *c* release and the molecular architecture of the cytochrome *c* conducting channel remain unknown. Previously, we demonstrated that apoptosis induction was accompanied by VDAC oligomerization, as revealed by cross-linking and directly monitored in living cells using Bioluminescence Resonance Energy Transfer technology. Moreover, apoptosis inhibitors inhibited VDAC oligomerization and a correlation between the levels of VDAC oligomerization and apoptosis was observed. Here, we combined site-directed mutagenesis with chemical cross-linking to reveal the contact sites between VDAC1 molecules in dimers and higher oligomers. Replacing hydrophobic amino acids with charged amino acids in b-strands 1,2 and 19, but not 14, interfered with VDAC1 oligomerization and apoptosis induction. Cysteine cross-linking results, from introducing cysteine at a defined position in cysteine-less VDAC1 and applying the cysteine-specific cross-linker, BMOE, supported the close vicinity of b-strands 1,2 and 19 in VDAC1 dimer. Moreover, the results suggest that VDAC1 exists as a dimer that undergoes conformational changes upon apoptosis induction to assemble into a higher oligomeric state. Additionally we demonstrated that the N-terminal region of VDAC1 lies inside the pore, but could also move and interact with the N-terminus from a second molecule to form a dimer. Our results suggest that the glycine rich sequence 21-GYGFG-25 is involved in the N-terminus translocation from the internal pore to the channel face. These results provide structural insight into cellular VDAC1's oligomeric state and its N-terminal region location and translocation.

202-Pos Board B2
The Influence of Membrane Physical Properties on Microvesicle Release in Human Erythrocytes
Laurie J. Gonzalez, Elizabeth Gibbons, Rachel W. Bailey, Jeremy Fairbourn, Thaothanh Nguyen, Samantha K. Smith, Katrina B. Best, Jennifer Nelson, Allan M. Judd, **John D. Bell**.
Exposure of human erythrocytes to elevated intracellular calcium causes fragments of the cell membrane to be shed as microvesicles. This study tested the hypothesis that microvesicle release depends on microscopic membrane physical properties such as lipid order, fluidity, and composition. Membrane properties were manipulated by varying the experimental temperature, membrane cholesterol content, and the activity of the trans-membrane phospholipid transporter, scramblase. Microvesicle release was enhanced by increasing the experimental temperature. Reduction in membrane cholesterol content by treatment with methyl-β-cyclodextrin also facilitated vesicle shedding. Inhibition of scramblase with R5421 impaired vesicle release. These data were interpreted in the context of membrane characteristics assessed previously by fluorescence spectroscopy with environment-sensitive probes such as laurdan, diphenylhexatriene, and merocyanine 540. The observations supported the following conclusions: 1) calcium-induced microvesicle shedding in erythrocytes relates more to membrane properties detected by diphenylhexatriene than by the other probes; 2) loss of trans-membrane phospholipid asymmetry is required for microvesicle release.

203-Pos Board B3
Apoptotic Effects of Nanoparticles on Human Cell Lines
Komal Vig, **Pooja M. Tiwari**, Michael E. Miller, Shivani Soni, Shree R. Singh.
Nanoparticles offer unique properties such as increased surface area and reactivity which allows them to easily translocate into cell membranes and enhance chemical reactions. It is therefore, important to understand their toxicity to the biological system before using them for any applications. In this study, we investigated apoptotic effects caused by nanoparticles mainly silver nanoparticle coated with PVP (10 nm) and gold nanoparticles (25 nm). We used a fluorescence marker 7-AAD (7- Aminoactinomycin D) which has strong affinity with DNA and intercalates in double-stranded DNA and has high affinity for GC-rich regions. It can be used for identification of necrotic/ late apoptotic cells and can be used as a marker for exclusion of nonviable cells. Cells were incubated with silver and gold nanoparticles at various concentrations and analyzed for apoptosis after periodic incubations. Our results indicate silver particles to cause more than 80% cell apoptosis at 25 μg/ml concentration. On the other hand, gold nanoparticles at the same concentration showed less than 10% cell apoptosis. As gold nanoparticles are relatively non-toxic to biological system they can be exploited for use in the medical therapy. Cells incubated with nanoparticles were also analyzed to determine their translocation into the cell. Ultrathin sections (5 μm) of the cells incubated with nanoparticles were cut and examined using TEM after negative staining. Initial studies indicate presence of nanoparticles in the vesicles of the cells and in the nucleus. We observed nanoparticle migration to nucleus also by confocal microscopy after 24 hr incubation which supported our TEM analysis. Work is currently on the way to determine the pathways of nanoparticle endocytosis by cell lines.

204-Pos Board B4
Study of Mitochondrial Dynamics During Apoptosis by Optical Fourier Filtering Detection
Robert M. Pasternack, Bryan Rabin, Neel J. Shah, Nada N. Boustany.
Recent evidence shows that changes in mitochondrial structure are associated with programmed cell death (apoptosis), and dysregulation of these changes is associated with human diseases including cancer, Parkinsons' disease and amyotrophic lateral sclerosis. Detecting morphological changes such as mitochondrial fission during the beginning stages of apoptosis is therefore of great interest. We have developed a light-scatter measurement technique that is sensitive to changes in mitochondrial structure in living apoptotic cells. This technique uses a spatial light modulator to achieve Fourier filtering using optical Gabor filters of dark field signal from cells and can be used without fluorescent dyes. We monitor mitochondrial fission in whole cells by detecting changes in object shape using an aspect ratio parameter generated from the optical Gabor information. Using our Gabor filtering methodology, we detect an average 15% decrease ($p < 0.05$) in the aspect ratio parameter of subcellular organelles at 60-100 min following apoptosis induction concomitant mitochondrial fragmentation confirmed by fluorescence microscopy. We have demonstrated that this decrease can be spatially registered with regions closely associated with the fluorescently labeled mitochondria and is absent in regions dominated by other organelles. The measurements may be made at low image resolution (>0.6 μm/pixel) and the data acquisition has recently been automated (nominal analysis of 100 single cells per second), offering significant potential for high throughput measurements. We are currently using the automated platform to study changes in mitochondrial dynamics during apoptosis at high time resolution. We will also investigate changes in mitochondrial membrane potential and their association with changes in mitochondrial structure measured with our technique in apoptosis-competent and apoptosis-resistant cells.

205-Pos Board B5
Analyzing Cellular Apoptosis Through Monitoring Mitochondrial Membrane Potential Changes with JC-10
Jinfang Liao, Qin Zhao, Xing Han, Zhenjun Diwu.
Apoptosis is one main type of programmed cell death which involves a series of biochemical events leading to specific cell morphology characteristics and ultimately death of cells. Mitochondrial membrane potential change is a key indicator of cell health and mitochondrial permeability transition is an important step in the induction of cellular apoptosis. JC-10, a lipophilic cationic dye, can be readily used to detect mitochondrial de-polarization. It has been successfully used in several different cell types (e.g., primary rat hepatocytes, CHO-K1, HeLa, Jurkat and HepG2 cell lines). JC-10 has improved water solubility. The consistent results were obtained with flow cytometry, fluorescence plate reader and florescence microscopy. In most of the cell lines JC-10 demonstrates excellent signal to background ratios. In addition, two other membrane potential-sensitive fluorescent dyes, the near infrared red Mito NIR, and orange color Mito Orange have also showed similar results as JC-10 for measuring mitochondria membrane potential changes in Jurkat and Hela cells. In conclusion JC-10, Mito NIR, and Mito Orange are robust tools for evaluating apoptosis targets and screening their agonists and antagonists with fluorescence microplate readers, fluorescence microscopes or flow cytometers.

206-Pos Board B6
Amphipathic Tail-Anchoring Peptide from BFL-1 and Pro-Apoptotic BH3 Peptides Induce Apoptosis Through Different Mechanisms
Jae-Kyun Ko, Kyoung-Han Choi, Jun Peng, Wei-Xing Zong, Noah Weisleder, Jialing Lin, Jianjie Ma.
Pro-apoptotic Bcl-2 homology domain-3 (BH3) peptides and mimetics have been developed as cancer therapeutics by targeting apoptotic regulators in cell death. Since tumor cells often contain altered expression levels of Bcl-2