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

Andrea Reinartz[1,2], Christopher A. Haynes[2], Ruth Knuechel[1], Alfred H. Merrill, Jr.[2], Nikolaus Gassler[1].
[1]RWTH Aachen University Hospital, Aachen, Germany, [2]Georgia Institute of Technology, Atlanta, GA, USA.

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

Lynn Anderson, Kelly Damm, Ryan Baker, Joseph Chen, Amy Hamaker, Izadora Izidoro, Eric Moss, Mikayla Orton, Kristin Papworth, Lyndee Sherman, Evan Stevens, Celestine Yeung, Jennifer Nelson, Allan M. Judd, John D. Bell.
Brigham Young University, Provo, UT, USA.

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

Jennifer Nelson, Erin Olson, Katalyn Griffith, Michael Streeter, Allan M. Judd, John D. Bell.
Brigham Young University, Provo, UT, USA.

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

Varda Shoshan-Barmatz, Shay Geula, Lior Aram, Nir Arbel.
Department of Life Sciences and the NIBN Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel.

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

Ulrika H. Englund, Jens Gertow, Fredrik Elinder.
IKE, Linkoping, Sweden.

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

frog Xenopus laevis during apoptosis. The two-electrode voltage-clamp technique was used to record endogenous ion currents in stage V or IV oocytes treated with staurosporine. We found that a sodium current was activated at voltages more positive than 0 mV with a mid point of the open-probability curve around +50 mV. Opening and closing kinetics were roughly exponential with time constants between 10 and 50 ms. The current was resistant to both 1 uM tetrodotoxin and 10 uM amiloride, while 200 uM verapamil in the bath solution completely blocked the current. Oocytes treated with both staurosporine and verapamil failed to upregulate the sodium current (measured in the absence of verapamil). We conclude that a verapamil-sensitive Na current is important in the apoptotic process in Xenopus oocytes.

2414-Pos

Photobiomodulation of Cellular Signalling and Apoptosis Induction in Human T Cells

Rahul P. Sinha, Gyongyver Katona, Magdalena Mocanu, Eugen Radu, Eva Katona.
Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

Aiming to contribute to the understanding of molecular and cellular mechanisms involved in photobiomodulation, the present studies were undertaken to monitor short and long term laser irradiation effects in metabolically intact and metabolically impaired human T cells. We used AlGaInP/GaAs lasers with emission wavelengths in the range 600 - 900 nm and exposed T leukemia lymphoblasts and peripheral blood derived adherent and non-adherent mononuclear cells, cultured in normal and in energy/nutrient restriction caused stress conditions, to doses and irradiation regimes of therapeutic significance (total incident doses up to 15 μJ/cell). Energy/nutrient restriction was realized by serum starvation, glucose deprivation or blockade of glycolysis/oxidative phosphorylation. Selecting appropriate molecular reporters, we traced changes occurring in characteristics of cell signaling key players, and rates of cellular proliferation and apoptosis induction. Cell cycle progression, percentage of apoptotic/necrotic cells, and intracellular calcium and ERK phosphorylation levels, were assessed in single cell and cell suspension measurements. The data obtained by conventional, phase contrast, and fluorescence microscopy, steady-state fluorimetry, electrophoresis/imunoblotting, and flow cytometry demonstrate significant cell type, cell state, irradiation regime, radiation dose, radiation wavelength, and treatment duration dependent soft laser effects in human T lymphocytes and leukemia lymphoblasts.
Partial financial support of the Romanian Ministry of Education, Research and Innovation (grant 42139/2008 "REUMALAS") is gratefully acknowledged.

DNA, RNA Structure & Conformation II

2415-Pos

Real-Time Detection of Cruciform Extrusion by Single-Molecule DNA Nanomanipulation

Ramreddy Tippana.
Institute of Jacques Monod, Paris, France.

Cruciform extrusion in dsDNA can occur when a DNA palindrome is subjected to physiological levels of negative supercoiling. Here we use single-DNA nanomanipulation to explore the kinetic and structural properties of cruciform extrusion induced by negative supercoiling. Cruciform extrusion appears as an abrupt increase in the extension of negatively supercoiled DNA, and the amplitude of the change in extension is proportional to the number of bases in the cruciform. The kinetics of this two-state system, B-DNA and cruciform DNA, can be tuned by negative supercoiling which destabilizes the former and stabilizes the latter. The rate of extrusion is controlled by the size of the apical loop, decreasing as the loop size is increased from 5 to 8 bases. Cruciform rewinding is controlled by features in the stem. Perfect cruciforms will tend to extrude irreversibly, whereas shortening and addition of imperfections to a stem can render extrusion reversible. From measurements of the effect of torque on extrusion/rewinding kinetics we propose that in the transition state to cruciform extrusion the palindrome is unwound in the unpaired loop region of the cruciform. These results provide insight into the mechanism of cruciform extrusion and help to understand the potential role of these structures in processes of genomic instability as well as those underpinning the synthesis of non-coding RNAs.

2416-Pos

A Direct Observation of Highly Bent and Twisted DNA at the Single Molecule Level

Troy Lionberger[1], Davide Demurtas[2], Todd Lillian[1], Julien Dorier[3], Noel Perkins[1], Andrzej Stasiak[3], Edgar Meyhofer[1].
[1]Univ. of Michigan, Ann Arbor, MI, USA, [2]Ecole Polytechnique Fédérale de Lausane, Lausanne, Switzerland, [3]Univ. of Lausanne, Lausanne, Switzerland.

Many DNA-binding proteins interact with twisted or bent DNA. To characterize the activity of these proteins as a function of the torsional and bending stresses, we must first understand how these mechanical stresses affect the DNA tertiary structure (topology). To experimentally define this relationship on scales that are biologically relevant to DNA-binding proteins requires DNA molecules which stably maintain high degrees of stress and deformation on a length scale appreciably below the persistence length. DNA minicircles of ~100bp in size offer a unique opportunity to achieve our required specifications. We have prepared circular DNA constructs (100bp, 106bp, and 108bp) sustaining comparable magnitudes of bending stress and varying degrees of torsional stress (which arises when linear DNA molecules of a non-integral number of helical turns are circularized). Using cryo-electron microscopy (cryo-EM) combined with 3-D image reconstruction, we have been able to quantitatively characterize the structural details at the molecular level of the topological effects of torsional stress within these minicircle constructs. We have observed the three species of minicircles under conditions of both weak and mild electrostatic repulsion, and measured the observed distributions of curvature (indicative of kink formation) and writhe (reflective of torsional stress). Despite the significant torsional stress sustained within the most highly stressed construct, all three are roughly planar, though the writhe and curvature distributions do depart significantly from theoretically predicted values. We are attempting to resolve the discrepancies between theoretical expectations and our observed experimental data using Brownian dynamics simulations of DNA minicircles sustaining varying degrees of torsional stress. We expect that this work will begin to define the behavior of highly stressed DNA at biologically relevant scales, and will broaden our understanding of how sub-persistence length DNA responds to mechanical stress.

2417-Pos

Measurement of the Elastic Energy of Sharply Bent Ds DNA

Hao Qu, **Yong Wang**, Chiao-Yu Tseng, Giovanni Zocchi.
UCLA, Los Angeles, CA, USA.

We present measurements of the elastic energy of short (30 bp), sharply bent, ds DNA molecules. The measurements are obtained by two independent methods: one is based on the monomer-dimer equilibrium of an appropriate configuration where the elastic energy stored in the bent strands drives dimer formation; the other is based on melting curves analysis. We find that, for example, the elastic energy of a sharply bent 30 bp double stranded DNA molecule with a nick at the center does not exceed 10 kBT.

2418-Pos

Visualizing and Quantifying the Energy Landscape during DNA Overstretching

Peter Gross[1], Niels Laurens[1], Lene B. Oddershede[2], Ulrich Bockelmann[3], Erwin J.G. Peterman[1], Gijs J.L. Wuite[1].
[1]VU University Amsterdam, Amsterdam, Netherlands, [2]Niels Bohr Institute, Copenhagen, Denmark, [3]ESPCI, Paris, France.

DNA undergoes a structural transition at a tension of 65 pN, were the polymer gains 70% of its contour length. The molecular basis of this overstretching transition has been elucidated using a combination of fluorescence microscopy and optical tweezers: At a tension of 65 pN, the DNA undergoes a nucleation-limited force-induced melting transition, in which the DNA strands gradually fray from the DNA's extremities during progression of overstretching [1].
Here we demonstrate that inhibition of this fraying process from one of the two DNA ends leads to a single deterministic melting front, which allows us to correlate the force signal in the overstretching plateau to the melted sequence.
We show that the propagation of the melting front progresses in bursts involving cooperative unbinding of multiple base-pairs. We furthermore proof that this burst-wise melting is an equilibrium process that is completely determined by the DNA sequence. Applying an equilibrium molecular stick-slip theory, we obtain a good agreement in both the force at which the DNA molecule starts to denature and the location of the individual melting bursts. We demonstrate that this theory, with the underlying DNA sequence as input, is able to predict the force-induced melting behavior.
Furthermore, we explore individual melting bursts by monitoring the force level of a DNA close to a melting event, and observe a bistability between two levels of the melting process. The time scale between the melting-reannealing events provides us with insight into the underlying local energy landscape close to a melting burst.
[1] van Mameren et al., Unraveling the structure of DNA during overstretching using multicolor, single-molecule fluorescence imaging, PNAS (in press) 2009

2419-Pos

Torsional Studies of DNA Denaturation using Angular Optical Trapping

Maxim Y. Sheinin[1], Scott Forth[1,2], Michelle D. Wang[1,2].
[1]Department of Physics, Cornell University, Ithaca, NY, USA, [2]Howard Hughes Medical Institute, Cornell University, Ithaca, NY, USA.

Torque-induced separation of duplex DNA strands plays an important role in a wide variety of cellular processes, such as transcription, DNA replication,