channels independently of Bax, the permeabilization is enhanced by the addition of less than 5nM oligomeric Bax. As much as 50nM oligomeric Bax alone did not result in any significant MOMP. The Bax enhancement occurs with an apparent affinity that increases with an increase in ceramide-induced MOMP, indicating an underlying mechanism by which Bax enhances ceramide-induced MOMP. Bax also causes apparent ceramide channel enlargement in yeast mitochondria, which lack Bcl-2 family proteins, as well as in planar phospholipid membranes, which is a defined, protein free, system. By contrast, monomeric Bax has no effect on ceramide channels in the aforementioned systems. The Bax inhibitor, Bci2 [Bruno Antonsson], prevents Bax mediated channel enlargement but does not affect permeabilization induced by ceramide alone. **Conclusions:** Both pro- and anti-apoptotic proteins regulate ceramide channels, consistent with ceramide channels being the pathway by which proteins are released by mitochondria early in apoptosis. (Supported by NSF grant: MCB-0641208)

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Bcl-2 Does Not Inhibit Bax Insertion During Intrinsic Apoptosis

Oscar Teijido Hermida, Kathleen W. Kinnally, Laurent M. Dejean.
New York University College of Dentistry, New York, NY, USA.

Mitochondrial outer membrane (MOM) permeabilization and cytochrome c release from mitochondria into the cytosol are considered to be the commitment steps of the intrinsic apoptotic pathway. Cytochrome c release is regulated by the Bcl-2 family proteins, which contains both pro-apoptotic (e.g. BAX) and anti-apoptotic (e.g. Bcl-2) members. It is now well established that after a death signal, cytosolic BAX is translocated to the mitochondrial outer membrane, inserted then in the double leaflet and activated through a conformational change. Activated BAX oligomerizes and might be associated to other mitochondrial proteins, leading to the formation of the Mitochondrial Apoptosis-induced Channel (MAC) into the MOM. This channel allows the release of cytochrome c into the cytosol. Bcl-2 inhibits MAC formation and therefore, cytochrome c release. However, the molecular mechanisms through which Bcl-2 affects earlier steps of BAX-mediated apoptosis are not fully understood.

In this study we investigated the effects of Bcl-2 over-expression on BAX-mediated apoptosis. We were able to confirm that Bcl-2 over-expression inhibits BAX translocation to the MOM and activation/oligomerization, as previously reported. Bax translocation is generally considered as the primary target of Bcl-2. Surprisingly, Bcl-2 over-expression did not alter the insertion status of BAX into the MOM. These data point out the further step, BAX activation/oligomerization, as the primary target of Bcl-2. Since Bcl-2 does not inhibit BAX insertion, we hypothesize that an event occurring after cytochrome c release triggers somehow a positive feedback on Bax expression and translocation. Thus, the inhibition of cytochrome c release by Bcl-2 could explain the further blocking on BAX translocation.

Supported by NYU Research Challenge Funds to LD.

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Estrogen-induced Protection of Heart Ischemia-reperfusion Injury by the Inhibition of the Mitochondrial Permeability Transition Pore (mPTP) in Isolated Heart Mouse

Jean C. Bopassa, Andrea Ciobotaru, Mansoureh Eghbali, Ligia Toro, Enrico Stefani.
UCLA School of Medicine, Los Angeles, CA, USA.

Although several studies have shown that the administration of 17ß-estradiol (E2) has a cardioprotective effect during ischemia-reperfusion (I/R), the mechanisms of this action are largely unknown. In this study, we investigated the effects of E2 on the opening of mPTP and as well on the myocardial infarct size after global myocardial I/R injury. Hearts of male mice were isolated and retrograde-perfused through aorta with the Langerdoff system at 37 oC. After 20 min of perfusion, hearts were subjected to 20min global ischemia followed by 40min reperfusion. Mitochondria were isolated to measure Calcium Resistance Capacity (CRC) and mPTP installation; infarct size was evaluated by triphenyltetrazolium chloride staining (TTC). Experiments were performed in hearts perfused with Krebs Henseleit solution or with Krebs Henseleit + E2 (100 pg/ml, corresponding to E2 peak concentration at proestrus). The E2-treated group had increased CRC (0.73+0.11 μM vs. 1.2+ 0.06, $p < 0.01$) and a reduced infarct size (43 ± 3% vs. 68 ± 5%, $p < 0.01$) compared to the control group. The E2-induced infarct size reduction was abolished by the specific estrogen receptor antagonist ICI 182,780 (100 nM). These results indicate that a brief E2 exposure favors CRC by inhibiting the mPTP installation resulting in a reduction of the infarct size. We propose these actions as a mechanism for E2-induced protection during I/R in isolated hearts.

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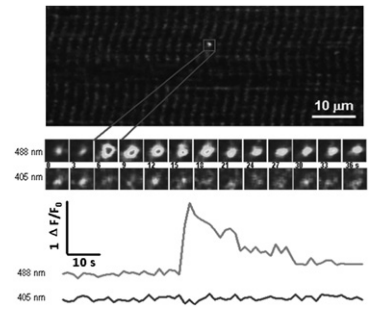
In vivo Imaging of Superoxide Flashes in Skeletal Muscle

Huaqiang Fang[1], Wanrui Zhang[1], Xianhua Wang[1], Wang Wang[2], Kaitao Li[1], Yanru Wang[1], Xiuqin Zhang[1], Shujiang Shang[1], Xiaoli Tian[1], Jingsong Zhou[3], Noah Weisleder[4], Jianjie Ma[4], Ming Zheng[1], **Min Chen[1]**, Heping Cheng[1].
[1]Institute of Molecular Medicine, National Laboratory of Biomembrane and Membrane Biotechnology, Peking University, Beijing, China, [2]Department of Integrative Biology and Physiology, University of Minnesota Medical School, Minneapolis, MN, USA, [3]Department of Molecular Biophysics and Physiology, Rush University School of Medicine, Chicago, IL, USA, [4]Department of Physiology and Biophysics, Robert Wood Johnson Medical School, Piscataway, NJ, USA.

Emerging evidence suggests that reactive oxygen species (ROS) constitute a class of signaling molecules that regulate diverse cell functions including metabolism, muscle contractility and apoptosis. Recently we have developed and characterized a highly sensitive and reversible superoxide-selective probe, a circularly permuted yellow fluorescent protein (cpYFP), and demonstrated quantal and transient superoxide-producing events (superoxide flashes) within single mitochondria across multiple cell types (Wang et al, *Cell*, 132, 279).To further understand the physiological significance of flash events, we generated the pan-tissue mt-cpYFP transgenic mice expressing cpYFP in the mitochondria of multiple tissues. *In vivo* imaging of superoxide signals in gastrocnemius of transgenic mouse under anesthesia revealed mitochondrial superoxide flashes with similar properties (Fig). Further, superoxide flashes were also visualized in isolated skeletal muscle fibers transfected *in vivo* by electroporation with mt-cpYFP. Our findings support that mitochondrial superoxide flash activity is a physiologically relevant phenomenon that may participate in diverse aspects of cell function and signaling.



2729-Pos Board B699

Visualization Of Mitochondria-targeted Photodynamic Effects Of Hpph-in Coupled With Visible Laser 637 Nm In Osteosarcoma 143b Cells

Mei-Jie Jou.
Chang Gung University, Tao-Yuan, Taiwan.

Photosensitizer HPPH cooperated with metal complex containing In (III) (HPPH-In) produces much efficient singlet oxygen production and photodynamic effects (PDE) as compared to HPPH alone. With the application of mitochondrial fluorescent probes and laser scanning imaging microscopy, mitochondrial level of PDE induced by HPPH-In coupled with visible laser 637 nm were investigated in detail. PDE of HPPH-In significantly enhances depletion of a mitochondria specific fluorescent probe MitoTracker Green at very earlier time points suggesting its primary targeting on the mitochondrial membrane. Mitochondria soon swelled and followed by plasma membrane blebing and later apoptotic condensation of nuclei and cell death. These mitochondria-associated apoptotic events induced by PDE of HPPH-In can be partially inhibited by a mitochondria antioxidant, MitoQ, and by the removal of extracellular Ca^{2+} suggesting a mROS- and Ca^{2+}-dependent mechanism is involved. When mitochondrial reactive oxygen species (ROS) formation and mitochondrial membrane potential depolarization ($\Delta\Psi$) were imaged simultaneously, PDE of HPPH-In significantly enhanced mROS formation and $\Delta\Psi$ depolarization with small delay. PDE of HPPH-In-induced increase in mROS soon propagated to adjacent non-irradiated mitochondrial population as well as that in adjacent cells and caused depolarization of$\Delta\Psi$ of these non-irradiated mitochondria. In addition to PDE of HPPH-In-enhanced mROS formation, we observed PDE of HPPH-In-induced sudden depolarization of $\Delta\Psi$effectively reduced mROS formation suggesting a possible protective preconditioning may exist. Finally, PDE of HPPH-In significantly altered mitochondrial dynamics by decreasing mitochondrial movement and enhancing fission of mitochondria. These observations suggest that multiple mitochondria-targeted devastating mechanisms provided by the PDE of HPPH-In coupled with 637 nm laser