mitochondrial membranes which result in the dramatic increase of their permeability. This increase in permeability during necrosis is caused by the opening of a Permeability Transition Pore - PTP in mitochondrial inner membrane. On the other hand apoptosis induces opening of a large non-selective channel (Mitochondrial Apoptotic Channel - MAC) in the mitochondrial outer membrane. We used Atomic force microscopy to study the structure and organization of native mitochondrial membranes isolated from necrotic and apoptotic cells. We complement our AFM imaging with nanopatricle-based immnunoassays, where mitochondrial membranes are pretreated with nanoparticle-conjugated antibodies for recognition of specific proteins. We will present our data on morphological studies of these membranes and will discuss feasibility of this approach for visualization of mPTP and MAC channels in the native mitochondrial inner membranes (MIM) and mitochondrial outer membranes (MOM) using AFM methods.

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Heterogeneity of Cardiolipin Regulation

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Cardiolipin (CL), is a protective phospholipid located exclusively on the mitochondrial inner membrane for stabilizing individual complex of mitochondrial respiratory chain for energy production, mitochondrial Ca2+ (mCa2+) regulation and reactive oxygen spices formation. Deficiency of CL has been associated with multiple mitochondrial dysfunctions upon pathological condition, diseases and aging. Using fluorescence digital imaging microscopy this study investigated how CL is preciously regulated in rat brain astrocytes RBA-1. Results demonstrate that at the resting stage, the distribution of CL is rather heterogeneous within single cell. The perinulear mitochondria show lower CL than mitochondria from the peripheral area. Heterogeneity of CL was observed even in single mitochondrion, which may affects mitochondrial fission and fusion mechanism. CL was found to be higher at the cross point area of mitochondrial network. Thread-like mitochondria and granular-like mitochondria, however, contain similar level of CL. Some of the thread-like mitochondria even contain lower CL than granular-like mitochondria. Upon localized oxidative stress induced by 488 nm laser irradiation, heterogeneous depletion of CL was observed among mitochondria populations within single cell. Distribution of CL altered also mitochondrial dynamics that depletion of CL always occurred before fission. Intriguingly, although complete depletion of CL is irreversible upon high strength of laser irradiation, depleted CL recovered during lower strength of laser irradiation and occurred heterogeneously within mitochondria populations. Condensation of CL was observed upon various stresses including oxidative stress induced by 488 nm laser irradiation, ionomycin-induced mCa2+ stress and arachidonic acid-induced lipid stress which crucially enhances preservation of CL and its resistance to stress which may be of significance for mitochondrial protection and survival. Unveiling pathophysiological regulation of CL thus may contribute significantly the prevention and treatment of CL associated pathological condition, diseases and aging.

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mtDNA T8993G Mutation-Induced Mitochondrial Complex V Inhibition Augments Cardiolipin-Dependent Alterations of Mitochondrial Dynamics During Oxidative, Ca2+ and Lipid Insults in NARP Cybrids: A Potential Therapeutic Target

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Mitochondrial dynamics including morphological fission and mitochondrial movement are essential to normal mitochondrial and cellular physiology. This study investigated how mtDNA T8993G (NARP)-induced inhibition of mitochondrial complex V altered mitochondrial dynamics in association with a protective mitochondrial phospholipid, cardiolipin (CL), as a potential therapeutic target. NARP cybrids harboring 98% of mtDNA T8993G genes and its parental osteosarcoma 143B cells were studied for comparison, and protection provided by melatonin, a potent mitochondrial protector, was explored. We demonstrate for the first time that NARP mutation significantly enhances apoptotic death as a result of three distinct lethal mitochondrial apoptotic insults including oxidative, Ca2+ and lipid stress. In addition, NARP significantly augmented pathological depletion of CL. NARP-augmented depletion of CL results in enhanced retardation of mitochondrial movement and fission and later swelling of mitochondria during all insults. These results suggest that CL is a common and crucial pathological target for mitochondrial apoptotic insults. Furthermore, CL possibly plays a central role in regulating mitochondrial dynamics that are associated with NARP-augmented mitochondrial pathologies. Intriguingly, melatonin, by differentially preserving CL during various stresses (oxidation > Ca2+ > lipid), rescues differentially CL-altered mitochondrial dynamics and cell death (oxidation > Ca2+ > lipid). Thus, melatonin, in addition to being a mitochondrial antioxidant to antagonize mitochondrial oxidative stress, a mitochondrial permeability transition modulator to antagonize mitochondrial Ca2+ stress, may stabilize directly CL to prevent its oxidization and/or depletion and, therefore, it exerts great potential in rescuing CL-dependent mitochondrial dynamics-associated mitochondrial pathologies for treatment of NARP-induced pathologies and diseases.

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Shuttling of Membrane-Bound Cytochrome B5 between Mitochondria and ER

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Cytochrome b5 type B has long been described as a tail-anchored protein of the outer mitochondrial membrane (cb5-mito). Using a fusion protein consisting of photoactivatable GFP fused to the C-terminus membrane-targeting segment of cb5-mito, we have found that while this construct concentrates in the mitochondrial membrane, it is continuously exchanged between the membranes of the mitochondrion and the endoplasmic reticulum. This effect does not depend on the presence of mitofusin 1 or 2, as it occurs in Mfn-null MEFs as well as in wild type cells. Interestingly, a point mutation in the hydrophobic, membrane-targeting sequence of cb5-mito that was recently reported to prevent its association with autophagosome membranes, also eliminates its exchange with the ER. While lipids and viral proteins have been shown to traffic from ER to mitochondria, this is, to our knowledge, the first demonstration of such a back-and-forth shuttling, representing both a new aspect of the ER-mitochondrial interface and an Mfn-independent pathway for the exchange of an outer mitochondrial membrane protein.

811-Pos Board B597

Dichotomy between Mitochondrial Ca2+ Uptake and Martix [Ca2+] in MICU1 Deficient Cells

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Mitochondrial calcium uptake is central to cell physiology, but the underlying molecular mechanism remains elusive. Recently, MICU1 was identified as a mitochondrial protein required for Ca2+ uptake but dispensible for mitochondrial respiration or membrane potential generation. To study the specific role of MICU1 in mitochondrial Ca2+ uptake, we used wild type HeLa cells (Ctrl), MICU1 knockdown by 2 distinct shRNAs (KD) and MICU1 rescued cells (Rescue). First, we monitored the cytoplasmic and mitochondrial matrix [Ca2+] ([Ca2+]c and [Ca2+]m, respectively) during store-operated Ca2+ entry. Specifically, cells were preincubated in a Ca2+-free buffer containing thapsigargin (Tg), to deplete the ER Ca2+ store, and then Ca2+ was added back and the ensuing [Ca2+]c (fura2) and [Ca2+]m (mitochondria-targeted pericams) changes were measured. In both Ctrl and KD a [Ca2+]c increase occurred that was associated with a [Ca2+]m rise only in Ctrl. The Rescue behaved similarly to the Ctrl. Also, when Ca2+ was added to permeabilized cells, no [Ca2+]m increase appeared in the KD. These results confirm that the shortage of MICU1 causes suppression of the [Ca2+]m signal. Surprisingly, when the Ca2+ entry was followed by uncoupler addition to release the mitochondrial Ca2+ content, a huge [Ca2+]c increase appeared in KD, However, when the uncoupler was added prior to Ca2+ entry, no [Ca2+]c increase occurred. Thus, mitochondria in the KD also accumulated Ca2+ during Ca2+ entry. Mitochondrial Ca2+ uptake evoked by Ca2+ addition was also documented in permeabilized KD by measurement of ruthenium red sensitive 45Ca uptake and cytoplasmic Ca2+ clearance (fura2). Thus, Ca2+ is accumulated by MICU1 deficient mitochondria but it fails to result an increase in [Ca2+]m. We are currently investigating, whether suppression of the [Ca2+]m rise in the KD is due to enhanced matrix Ca2+-buffering.

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MICU1 Serves as a Ca^{2+}-Controlled Gatekeeper for the Mitochondrial Ca^{2+} Uniporter

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MICU1 serves as a Ca^{2+}-controlled gatekeeper for the mitochondrial Ca^{2+} uniporter Mitochondria are important targets and relay points of calcium signaling. Ca^{2+} is driven to the mitochondrial matrix via a low-affinity Ca^{2+} channel, the Ca^{2+} uniporter (MCU) that exhibits time-dependent