Mitochondria in Cell Life and Death

3056-Pos Board B486
New Fluorescence Probes for Visualizing Cell Structures and Function

Yuning Hong
School of Chemistry, University of Melbourne, Parkville, Australia.
Organic fluorogens with aggregation-induced emission (AIE) characteristics have demonstrated their potential to be ideal candidates for live cell imaging. Opposite to conventional organic dyes, the AIE luminoogens are non-luminescent when molecularly dissolved but highly emissive upon aggregation. As small molecules, the AIE luminoogens normally enter cells through diffusion, accumulate in the target location, and generate light emission. Inherently, they possess large Stokes shift (> 100 nm) with appreciable brightness and they are resistant to photo-bleaching and blinking, owing to the formation of aggregates inside the cells. In addition, they are structurally simple and synthetically accessible: the excitation/emission wavelengths as well as the functionalities can be fine-tuned via structural modification. In this study, a series of AIE dyes have been constructed and their applications for specific imaging of different organelles, tracking dynamics of mitochondria as well as sensing intracellular environment in physiological and pathological conditions will be introduced.

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Monitoring Mitochondrial Membrane Potential with Mitoview 633: A New Molecular Probe

Jarod Benowitz1,2, Qince Li1, KahYong Goh1, Chih-Chang Wei1, Luang Zhou1,2
1Physics, Utah State University, Logan, UT, USA, 2Cardiovascular Disease, University of Alabama at Birmingham, Birmingham, AL, USA, 2Biomedical Engineering, University of Alabama at Birmingham, Birmingham, AL, USA.
Background: Noninvasive mitochondrial inner membrane potential (ΔΨm) measurement has been largely constrained to the use of fluorescent probes. These probes accumulate in the mitochondrial inner membrane according to the Nernst equation. In this manner, ΔΨm can be measured indirectly by the intensity of the fluorescent dye. Objective: MitoView 633 is a new fluorescent probe in the far-red spectrum, which has great potentials in bioenergetics studies. However, its application in live cell imaging has not been well characterized. We aimed to determine the spectrum of MitoView using a spectrophotometer and characterized its dynamics in cardiomyocyte during live cell imaging.
Method: H9C2 cells or adult rat cardiomyocytes were stained with MitoView or TMRM or MitoSOX and far-red fluorescence was used to induce mitochondrial depolarization and the change of ΔΨm was recorded using a confocal microscope. Imagines were processed using ImageJ and the dynamics of MitoView and TMRM were compared. In some experiments, cells were co-stained with MitoSOX for simultaneous ROS and ΔΨm recording.
Results: Spectrum analysis showed that MitoView emission could be detected at 660 ± 50 nm. In addition, MitoView perfectly located in mitochondrial matrix, as demonstrated by its colocalization with Mito-YFP or TMRM in H9C2 cells and rat cardiomyocytes. Furthermore, the photobleaching of MitoView was insignificant and comparable to that of TMRM and the FCCP-induced decay of MitoView fluorescence was faster than TMRM, indicating its superior sensitivity to ΔΨm. Finally, MitoView could be co-stained with MitoSOX, allowing for simultaneous measurement of ROS and ΔΨm in live cell imaging.
Conclusion: We showed that MitoView is very sensitive to ΔΨm. Due to its minor photobleaching effect, high sensitivity to ΔΨm, and far-red emission spectrum, MitoView could be an ideal molecular probe to monitor ΔΨm alone or together with other indicators in live cell imaging.

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Biophysical and Biochemical Properties of the Large Conductance Potassium Channel in Fibroblast Mitochondria

Piotr Bednarczyk1,2, Anna Kicińska1, Wiesława Jarmuszkiewicz3,2,3
1Physics, Utah State University, Logan, UT, USA, 2Cardiovascular Disease, University of Alabama at Birmingham, Birmingham, AL, USA, 3Department of Biophysics, Warsaw University of Life Sciences - SGGW, Warsaw, Poland.
Background: Potassium channels have been found in the inner mitochondrial membranes of human fibroblast. Potassium channel opener BMS191095 and diazoxide. The influence of substances modulating ATP-regulated potassium channel activity on the bioenergetics, oxygen consumption and membrane potential, of isolated human dermal fibroblasts was investigated. Since aberrant mitochondrial dynamics plays key roles for the pathogenesis of cardiovascular disease, we investigated the relationship between EPA-mediated cardiac protection and mitochondrial dynamics. Methods and Results: When differentiated H9c2 myocytes were exposed to palmitate (PAL; 400 μM, saturated fatty acid) for 24 hours, the PAL-treated myocytes exhibited depolarized mitochondrial membrane potential (ΔΨm) measured with JC10 ratio; 2.1 ± 0.4 vs. 4.5 ± 0.3 of control, P < 0.01) and activated caspase3/7 (measured with luminescent assay; 4.1 ± 0.7 fold increase from control, P < 0.01), suggesting the PAL-induced lipotoxicity. The PAL-treated myocytes also showed dynamin-related protein 1 (Drp1) upregulation and fragmented mitochondria (75.3 ± 5.0 % of cells vs. 9.2 ± 3.2 % of control, P < 0.01), indicating aberrant mitochondrial dynamics by PAL. When the PAL-treated myocytes were co-incubated with EPA (50 μM), the caspase3/7 activation by PAL (1.5 ± 0.3 fold increase vs. PAL, P < 0.01) was attenuated, and the ΔΨm determination by PAL was restored (2.9 ± 0.2 vs. PAL, P < 0.01). EPA also suppressed the PAL-induced Drp1 expression and mitochondrial fragmentation (37.6 ± 6.4% vs. PAL, P < 0.01). In addition, EPA alone reduced the Drp1 expression and exhibited elongated mitochondria. Conclusion: We conclude that EPA ameliorates the PAL-induced lipotoxicity by the regulation of mitochondrial dynamics through Drp1.

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