Detection of Reactive Oxygen Species (ROS) in Live Cell Mitochondria

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Introduction

Oxidative stress in mitochondria has received intensive attentions recently because it is the most important source of intracellular reactive oxygen species (ROS) and is considered as a major contributor to some public health-related diseases, such as asthma, atherosclerosis, diabetic vasculopathy, osteoporosis, a number of neurodegenerative diseases and Down's syndrome. However, the existing probes have difficulties to target live cell mitochondria and distinct the individual ROS.

We have developed a new family of mitochondrial ROS fluorescence probes, which not only can specifically localize in mitochondria, but also can selectively detect the different ROS species, including superoxide, hydroxyl radical and hydrogen peroxide in multiple Ex/Em wavelengths.

Cancer cells stained with the novel fluorescent probes showed negligible fluorescence in absence of ROS stimulants. In contrast, OxiVision Blue-loaded cells with hydrogen peroxide treatment displayed strong blue fluorescence in mitochondria. MitoROS OH580-loaded cells with Fenton reaction (to induce hydroxyl radical) showed strong red fluorescence in mitochondria. MitoROS 520-loaded cells exhibited strong green fluorescence in mitochondria after treated with superoxide stimulants. All these probes have also been successfully used with a flow cytometer for the quantitative detection of ROS in live cells.

In conclusion, these probes are mitochondrion-targeted ROS probes, which can be used for monitoring the exogenous and endogenous changes of ROS levels in living cells in real time by fluorescence imaging and flow cytometer.

Material and Methods

Cell Line:

Cervical cancer HeLa cells and RAW 264.7 macrophage cells were seeded overnight in a 96-well black wall/clear bottom costar plate at 37 ºC in DMEM. Non-adherent cell line Human T lymphocyte Jurkat cells were grown at 37 ºC in RPMI 1640.

Cell Treatment:

Dye-loaded cells were treated with hydrogen peroxide, Fenton reaction, PMA (phorbol 12-myristate 13-acetate), Antimycin A (AMA) and Pyo (Pyocyanin) to generate intracellular hydrogen peroxide (H_2O_2), exogenous & endogenous hydroxyl radical (•OH), and exogenous & endogenous superoxide ($O_2^{\bullet-}$), respectively.

Fluorescence Probe	Target ROS/RNS	Ex/Em (nm)	Fluorescence Microscope /Flow Cytometer Channel
OxiVision Blue	H_2O_2 (mitochondria)	405/450	DAPI
MitoROS OH580	•OH (mitochondria)	576/598	TRITC
MitoROS 520	$O_2^{\bullet-}$ (mitochondria)	509/534	FITC
MitoROS 580	$O_2^{\bullet-}$ (mitochondria)	540/590	TRITC

Mitochondrial H_2O_2 Detection by OxiVision Blue

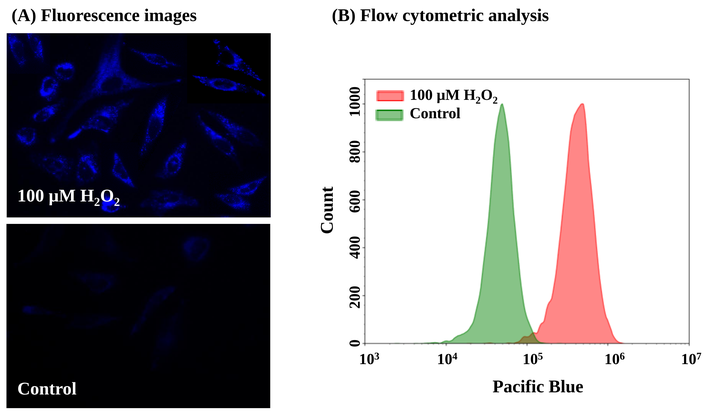

Figure 1. Fluorescence images of mitochondrial hydrogen peroxide (H_2O_2) in HeLa cells (A), and flow cytometric analysis of Jurkat cells (B) stained with OxiVision Blue for 30 minutes and treated with or without 100 μM H_2O_2 at 37 ºC for 90 minutes. The fluorescence images were acquired using fluorescence microscope with a DAPI filter. Flow cytometric analysis were obtained using ACEA NovoCyte flow cytometer in Pacific Blue channel.

Imaging Mitochondrial •OH by MitoROS OH580

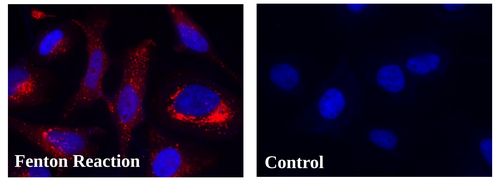

Figure 2. Fluorescence images of mitochondrial hydroxyl radical (•OH) in HeLa cells. Cells stained with MitoROS OH580 were treated with or without Fenton reaction (10 μM $CuCl_2$ and 100 μM H_2O_2) at 37 ºC for 1 hour to induce exogenous hydroxyl radical. Red: MitoROS OH580; Blue: Hoechst 33342.

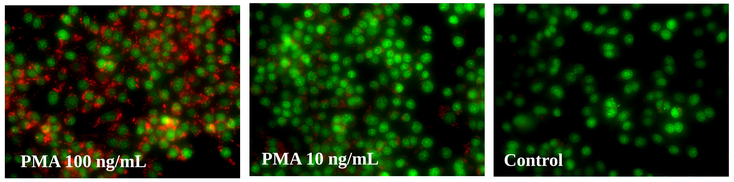

Figure 3. Fluorescence images of mitochondrial hydroxyl radical (•OH) in RAW 264.7 macrophage cells. Cells stained with MitoROS OH580 were treated with PMA (phorbol 12-myristate 13-acetate) in the range of 0-100 ng/mL in growth medium at 37 ºC for 4 hours to stimulate endogenous hydroxyl radical. Red: MitoROS OH580; Green: Nuclear Green LCS1.

Mitochondrial $O_2^{\bullet-}$ Detection by MitoROS 520, MitoROS 580

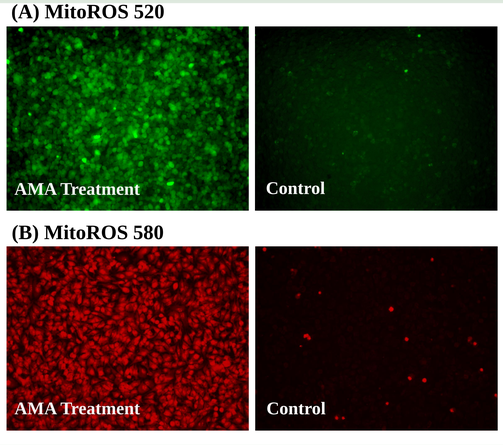

Figure 4. Fluorescence images of mitochondrial superoxide ($O_2^{\bullet-}$) in RAW 264.7 macrophage cells stained with (A) MitoROS 520 or (B) MitoROS 580 with or without 50 μM AMA (Antimycin A) treatment at 37 ºC for 30 minutes. (C) HeLa cells seeded overnight in a 96-well black wall/clear bottom plate, were stained with MitoROS 580 and incubated with 50 μM Pyo (Pyocyanin), 50 μM AMA or without treatment (Control) at 37 ºC for 30 minutes. The fluorescence signal were monitored at Ex/Em = 540/590 nm (cutoff = 570 nm) with bottom read mode using a CLARIOstar microplate reader (BMG Labtech). (D) Flow cytometric analysis of MitoROS 580 labeled Jurkat cells treated with or without 50 μM AMA at 37 ºC for 1 hour. The fluorescence signal was monitored at FL2 channel using a flow cytometer (BD FACSCalibur).