Mitochondrial function evaluation on UVB-exposed keratinocytes measured using fluorescent probes on the Sapphire Biomolecular Imager

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Introduction

One of the major effects of ultraviolet B (UVB) radiation is the induction of apoptosis on exposed cells, characterized by morphological changes such as chromatin condensation, nucleus fragmentation, contraction of cytoplasmic volume, and generation of apoptotic bodies containing intact organelles (1). Direct UVB-induced DNA damage stimulates p53 activation to repair the genotoxic injury. If the damage is too extensive to be repaired, p53 activates a cascade of events that includes alterations to the mitochondrial electron transport chain, leading to mitochondrial depolarization, and eventually culminates in apoptotic cell death (2).

Reactive oxygen species (ROS) are generated at low levels during normal metabolism and have been shown to play important roles as second messengers. However, in UVB-exposed cells, higher amounts of ROS are produced, mainly due to incomplete oxygen reduction in the mitochondria (3). ROS include singlet oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radical, which have been implicated in skin inflammation and cancer. These oxidative species are regulated by a group of antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, and catalase, whose expression is regulated by different stimuli. When the antioxidant response is overwhelmed, oxidation of proteins, DNA, and lipids occurs, leading to deleterious effects on cells and therefore contributing to UVB-induced apoptosis.

This application note presents the use of two fluorescent probes (green $DiOC_6$ and red MitoSOX) to evaluate mitochondrial alterations in UVB-exposed keratinocytes (4).

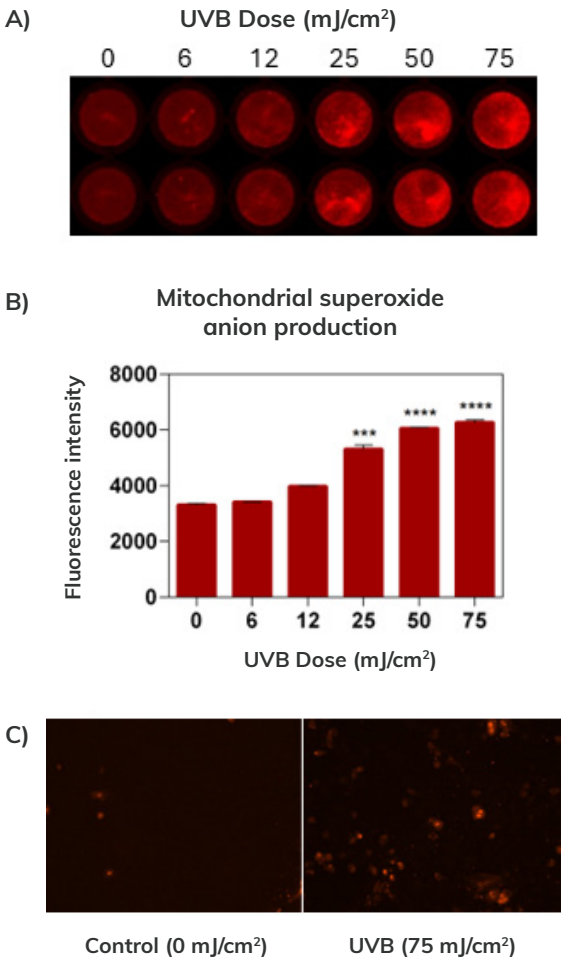

Figure 1. Mitochondrial superoxide anion production in UVB-exposed keratinocytes.

A) Fluorescence image of mitochondrial superoxide anion determination in 96-well plate obtained with Azure Sapphire Biomolecular Imager, using excitation and emission wavelengths of 520/565 nm. B) Fluorescence analysis was performed with ImageJ software. Bar graph shows the quantification of the plate presented in A. C) Fluorescence microscopy images of representative control and irradiated (75 mJ/cm²) cells obtained in an EVOS imaging system (Life Technologies).

Materials and Methods

Human keratinocyte cell line (HaCaT) was used for the experiments. HaCaT cells were cultured in Dubelcco's modified Eagle's medium, high glucose (DMEM-HG; Gibco-BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum and an antibiotic solution (PSG; Gibco-BRL). Cells were irradiated using an 8W UVM-28 Mid-Range Wave (302 nm) lamp from Ultraviolet Products (UVP, Upland, CA, USA). Dihexyloxacarbocyanine iodide ($DiOC_6$, $\lambda_{excitation}$ = 484 nm; $\lambda_{emission}$ = 511 nm) (Merck) was used to evaluate mitochondrial membrane potential. MitoSOX red ($\lambda_{excitation}$ = 500 nm; $\lambda_{emission}$ = 610 nm; from Molecular Probes, Thermo Fisher) was used to evaluate mitochondrial superoxide anion production.

Experimental procedures included the following steps:

1. HaCaT cells were seeded on 96-flat clear plastic well plates at a density of $5x10^5$ cells/ml in complete medium. To promote cell adherence to surface, cells were cultured during 48h at 37°C, 5% CO_2.

2. Cells were washed with phosphate-buffered saline (PBS) and irradiated with the UVB lamp, using six radiation doses: 0, 6, 12, 25, 50, and 75 mJ/cm^2. After exposure, the wells were filled with cell culture medium and the plates were left for 24h at 37°C, 5% CO_2.

3. Without removing the culture medium, $DiOC_6$ (30 nM) or MitoSOX (5 µM) were added to each well and incubated for 30 minutes at 37°C, 5% CO_2.

4. The plate was scanned on the Azure Sapphire Biomolecular Imager at the highest speed using the 488 channel for $DIOC_6$ probe and the 520 channel for MitoSOX. The intensity was set to 3 and the pixel size was 100 µm.

5. Images were analyzed using ImageJ to quantify the fluorescence intensity in each well.

Results

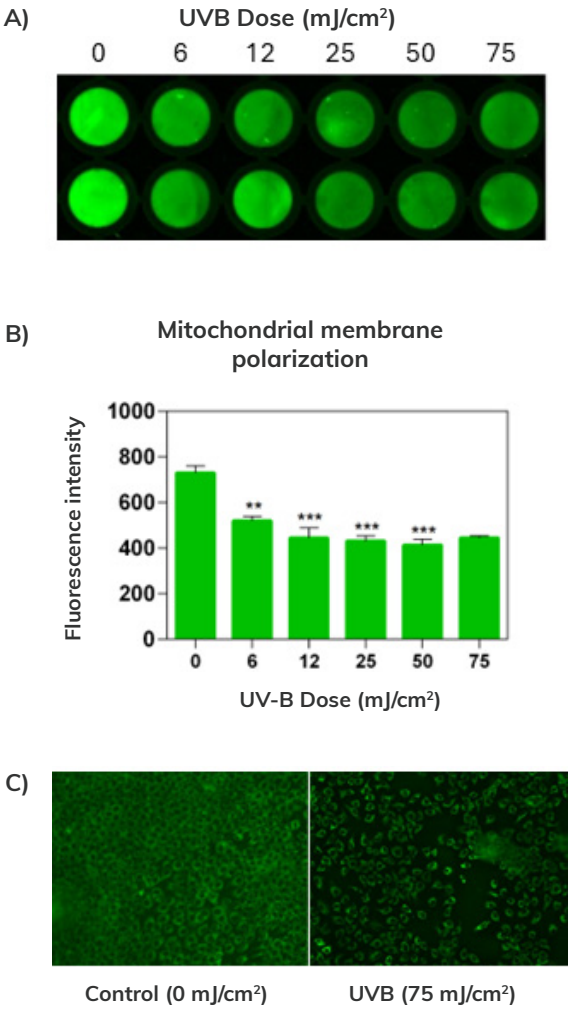

Figure 2. Mitochondrial membrane polarization in UVB-exposed keratinocytes.

A) Fluorescence image of mitochondrial membrane polarization evaluated in a 96-well plate, captured with Azure Sapphire Biomolecular Imager (excitation and emission wavelengths of 488/518 nm). B) Fluorescence analysis performed with ImageJ software. Bar graph shows the quantification of the plate presented in A. C) Fluorescence microscopy images of representative control and irradiated (75 mJ/cm^2) cells obtained in an EVOS imaging system (Life Technologies).

Conclusion

The Sapphire Biomolecular Imager allows the detection of fluorescence emitted by adherent cells grown in clear plastic well plates incubated with MitoSOX and $DiOC_6$ probes. It was demonstrated that the fluorescence intensity detected in the wells using the MitoSOX probe increased as the radiation dose increased, indicating a significant rise in mitochondrial superoxide anion production. For cells incubated with the $DiOC_6$ probe, the opposite effect was observed. The fluorescence intensity decreased as the radiation dose increased, indicating mitochondrial membrane depolarization. Both results indicate that Azure Sapphire Biomolecular Imager is useful to measure mitochondrial function as an indicator of the deleterious effects on cells caused by UVB radiation.

Acknowledgements

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References

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