Morphological Characterization of Photodynamic Therapy

Dana Darvish[1], Michal Safrai[1], Zvi Maik[1] and Aryeh Weiss[2]
[1]Faculty of Life Sciencs and [2]School of Engineering
Bar Ilan University, Ramt-Gan, Israel.

Introduction: Photodynamic therapy (PDT) is a promising technique based on using light to treat cancer. PDT requires the presence of a photosensitizer, light and oxygen. The light energy absorbed by the photosensitizer produces reactive singlet oxygen, resulting in local photo-oxidation, which kills the cells [2]. Malignant melanoma is the lethal skin cancer, and causes 1 out of 3 skin cancers deaths. Conventional chemotherapy has almost no effect on melanoma. When treated with the photosensitizer prodrug 5 aminolevulinic acid (ALA), B16 melanoma cells express increased Protoporphyrin IX (PpIX) , resulting in the photoinduced cell death[3].

In the present study, we use live cell time lapse microscopy to investigate the development of morphological changes in B16 cells which have been incubated with 1mM ALA, and then exposed to light (30 sec irradiation with 470nm excitation from the microscope fluorescence illuminator). Morphological changes are widely used to detect apoptosis, but generally only the end points of the process are observed. We are attempting to use morphological analysis to follow the onset and time course of the process.

Methods: B16 melanoma cells were incubated with 1mM ALA for either one, two, three or four hours. The control had ALA added immediately prior to timelapse imaging (no incubation). They were then irradiated with blue light (470nm +/- 20nm) for 30 seconds.

Live cell timelapse imaging was done on a Nikon TE2000E inverted microscope with a 40x/NA=0.6 LWD objective (since the cells were in plastic culture dishes). An on-scope incubator (Life Imaging Services, Olten, Switzerland) maintained temperature and CO_2 concentration. This setup has been able to maintain cells with no apparent ill effects for at least three days. An XCite 120W metal halide lamp provided the blue excitation at a setting of 25% (higher excitations killed the cells too quickly) using a 470nm +/- 20nm bandpass filter. The images were acquired with an Optronics single chip color CCD video camera, using the NIS elements software package to control the entire apparatus. The images were acquired over one hour.

Results: While the cells demonstrated clear morphological changes associated with apoptosis, the time course of these changes are not simple to describe. Dendrites progressively shrank, while the nuclei first appeared to swell and then shrink relative to the total cell area. There were changes in texture, as expected. Changes appeared to occur more quickly after 4 hours ALA incubation, but the two hour incubation was sufficient to bring the cells to a similar state (although perhaps a bit slower). Following one hour incubation, similar effects were observed, but after one hour the cells still appeared viable. Addition of ALA just prior to exposure and observation, with no incubation, did not result in changes in cell morphology or cell death. Time sequences of the cells for different incubation times are shown in Figure 1.

Conclusions: The usual morphological indicators of apoptosis (e.g., shrinking of the nucleus), may not be useful for studying the time course of apoptosis or necrosis. We plan to develop quantitative morphological measures to characterize the effects of PDT on B16 melanoma cells. Possible measures include: concavity, to detect changes in dendritic morphology; roundness, both of the cell and of the nucleus; and texture measures, to detect blebbing. In addition, we are developing dynamic measures designed to look at changes in cell motility, and rapid changes in shape. Finally, we will be doing these studies over longer time spans, once we are equipped to run multiple experiments in parallel using multiwell plates and a motorized XY stage.

References

1. Ickowicz Schwartz D, Gozlan Y, Greenbaum L, Babushkina T, Katcoff DJ, Malik Z., Differentiation-dependent photodynamic therapy regulated by porphobilinogen deaminase in B16 melanoma. Br J Cancer. 2004 May 4;90(9):1833-41.
2. Uzdensky A, Kolpakova E, Juzeniene A, Juzenas P, Moan J. The effect of sub-lethal ALA-PDT on the cytoskeleton and adhesion of cultured human cancer cells. Biochim Biophys Acta. 2005 Feb 11;1722(1):43-50. Epub 2004 Dec 15.
3. Peng Q, Warloe T, Berg K, Moan J, Kongshaug M, Giercksky KE, Nesland JM. 5-Aminolevulinic acid-based photodynamic therapy. Clinical research and future challenges. Cancer. 1997 Jun 15;79(12):2282-308. Review.

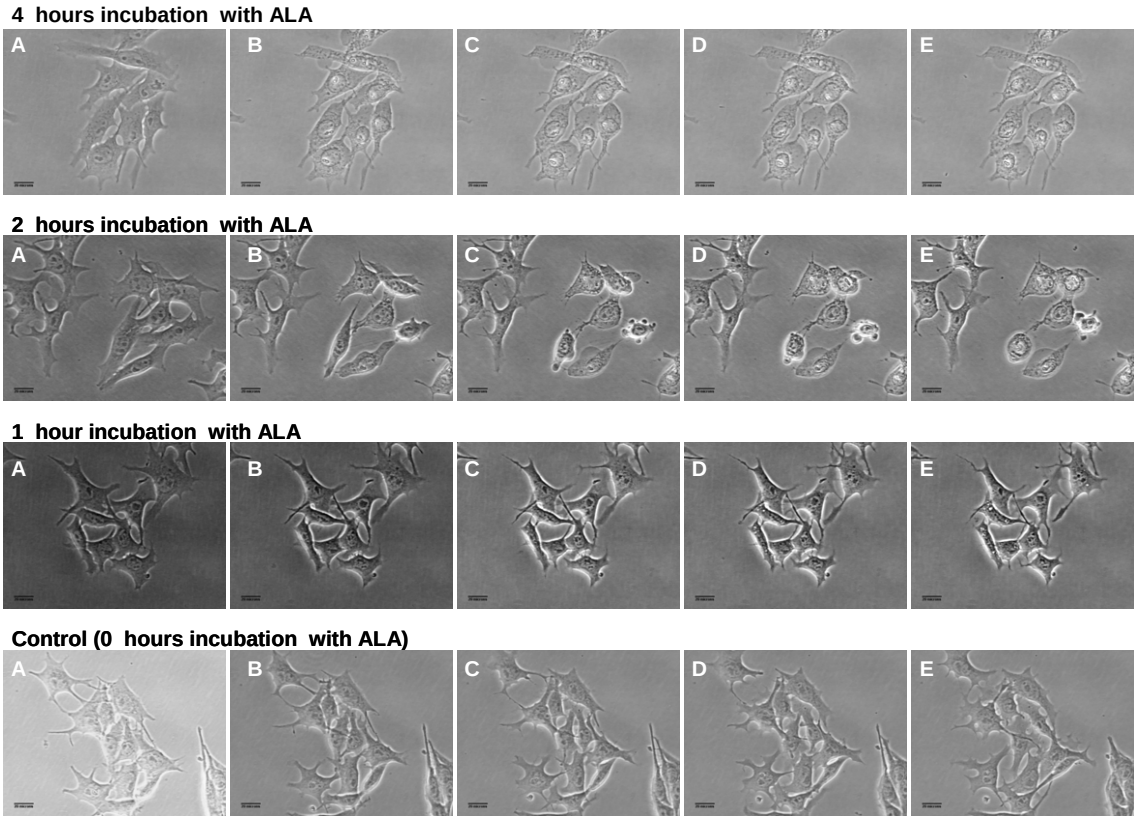

Figure 1 Morphological changes of B16 melanoma following 5-ALA-PDT at 4 different ALA incubation times. (A) Before exposure to blue light. (B-E) 6, 12, 24, 60 minutes after PDT respectively. The scale bar in the lower left corner is 20µm.