Bystander responses to photodynamic therapy: critical involvement of gap junction communication, calcium and nitric oxide signaling

Bianca Calì[1,2,] Stefano Ceolin[3,] Federico Ceriani[1,3], Mario Bortolozzi[1,3], Andrielly H.R. Agnellini[1,2,], Veronica Zorzi[1,3], Andrea Predonzani[4], Vincenzo Bronte[1,5 *], Barbara Molon [4a*], Fabio Mammano[1,3,6,a,*]

1. Foundation for Advanced Biomedical Research, Venetian Institute of Molecular Medicine, I-35129 Padua, Italy
2. University of Padua, Department of Surgery Oncology and Gastroenterology, Oncology and Immunology Section, I-35128 Padua, Italy
3. University of Padua, Department of Physics and Astronomy, I-35131 Padua, Italy
4. IRCCS, Istituto Oncologico Veneto, I-35128 Padua, Italy
5. Verona University Hospital, Department of Pathology and Diagnostics, Immunology Section, I-37134 Verona , Italy
6. (present address) CNR, Institute of Cell Biology and Neurobiology, I-00015 Monterotondo (RM), Italy

(a) These authors contributed equally to this work

e-mail: fabio.mammano@cnr.it

Keywords: Cancer, photodynamic therapy, nitric oxide, calcium signaling, connexins, apoptosis.

The phrase “bystander effects” was initially adopted in a radiotherapy context to account for responses observed in cellular systems that have not been directly traversed by ionizing radiations but are in close proximity to irradiated cells [1,2]. Bystander effects triggered by ionizing radiations in tumor and tumor-infiltrating cells include altered gene expression, DNA damage, mutation, malignant transformation and cell death. Bystander responses have been observed also as a consequence of other insults including ultraviolet radiation, heat, chemotherapy agents and photodynamic therapy; however the underlying mechanism and role in clinically relevant scenarios remain incompletely defined [1,2].

Here we show that activation of the commercially available and well-characterized photosensitizer AlClPc [3,4,5] in a single cell triggers apoptosis in bystander cancer cells. We demonstrate that cells are electrically coupled by gap junction channels and support the propagation of a Ca^{2+} wave initiated in the irradiated cell. The latter also acts as source of nitric oxyde (NO) that diffuses to bystander cells, in which NO levels are further increased by a mechanism compatible with Ca^{2+}-dependent enzymatic production. We detected similar signals in tumors grown in dorsal skinfold chambers applied to live mice, suggesting that the underlying signaling mechanisms are relevant for in vivo photodynamic therapy. Pharmacological blockade of connexin channels significantly reduced the extent of apoptosis in bystander cells, consistent with a critical role played by intercellular communication, Ca^{2+} and NO in the bystander effects triggered by photodynamic therapy [6].

[1] K.M. Prise, J.M. O'Sullivan, Nat Rev Cancer 9 (2009) 351-360.
[2] K.T. Butterworth et al., Clin Oncol (R Coll Radiol) 25 (2013) 586-592.
[3] I. Rosenthal, Photochemistry and Photobiology 53 (1991) 859-870.
[4] M. Kyriazi, et al. Photodermatol Photoimmunol Photomed 24 (2008) 87-94.
[5] N. Sekkat, et al. Molecules 17 (2012) 98-144.
[6] B. Cali, et al. Oncotarget (2015) published on-line 25 Mar 2015.

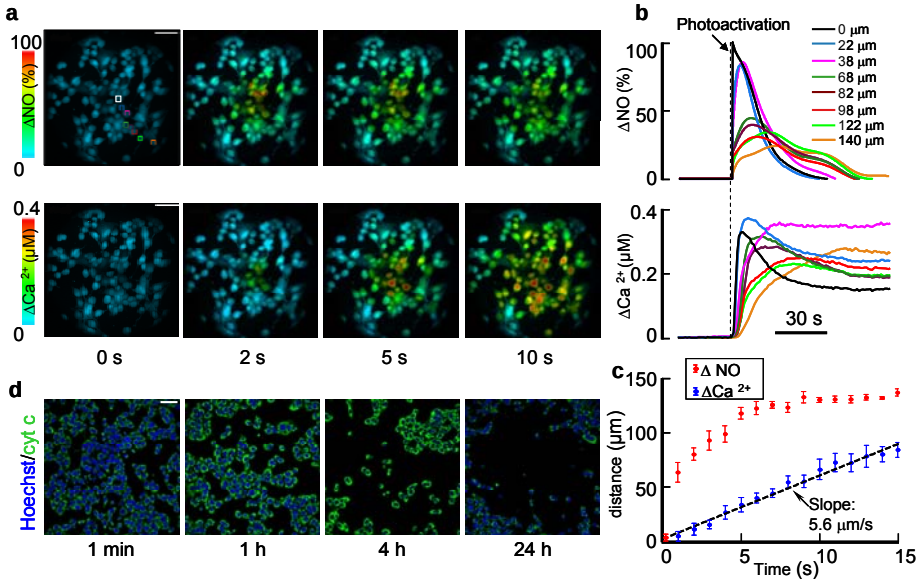

Figure 1. Focal photodynamic injury, i.e. photo–activation of the photosensitizer AlClPc for 60 s in a single cell of a C26GM mouse colon carcinoma cell culture, triggers NO and Ca^{2+} signals that depart from the irradiated cell and rapidly invade bystander cells; these events are followed by cytochrome c release and widespread cell death. (**a**) Representative false–color images of simultaneously recorded cytosolic NO (top) and Ca^{2+} (bottom) concentration changes (Δ) during focal photodynamic injury; the irradiated cell is encased in a white region of interest (ROI); scale bar, 50 μm. (**b**) Single-cell fluorescence traces obtained as pixel averages from the corresponding (color-matched) ROIs in (a); irradiated cell responses are shown as black traces; the vertical dashed line marks the onset of laser irradiation; ΔNO data were normalized to the corresponding maximal response in the irradiated cell (see Methods); (**c**) The distance at which bystander cell signals reach 50% of their first peak amplitude is shown as a function of time after the onset of focal photodynamic injury. Data are mean ± s.e.m. from n = 6 cultures; the dashed line is a least square linear fit with a slope of 5.6 μm/s. (**d**) Cultures were rapidly fixed at shown time points after focal photodynamic injury and immunostained with a cytochrome c antibody and the nuclear counter stain Hoechst; note that images in (d) are from different cultures, whereas those in (a) are all from the same culture; scale bar, 25 μm.