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Addenda

Initiation of Apoptosis and Autophagy by Photodynamic Therapy

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ABSTRACT

This study was designed to examine modes of cell death after photodynamic therapy (PDT). Murine leukemia L1210 cells and human prostate Bax-deficient DU-145 cells were examined after PDT-induced photodamage to the endoplasmic reticulum (ER). Previous studies indicated that this treatment resulted in a substantial loss of Bcl-2 function. Both apoptosis and autophagy occurred in L1210 cells after ER photodamage with the latter predominating after 24 hr. These processes were characterized by altered cellular morphology, chromatin condensation, loss of mitochondrial membrane potential and formation of vacuoles containing cytosolic components. Western blots demonstrated processing of LC3-I to LC3-II, a marker for autophagy. In DU145 cells, PDT initiated only autophagy. Phosphatidylinositol (PI) 3-kinase inhibitors suppressed autophagy in both cell lines as indicated by inhibition of vacuolization and LC3 processing. Inhibitors of apoptosis and/or autophagy were then used to delineate the contributions of the two pathways to the effects of PDT. Given the ability of autophagy to upregulate MHC-11 peptide presentation, autophagy may play a role in the ability of photodynamic therapy to stimulate immunologic recognition of target cells.

Photodynamic therapy (PDT) involves the photosensitization of neoplastic cells and tissues with porphyrins or related structures that catalyze, upon irradiation, formation of reactive oxygen species.¹ While effects of PDT on tissues also include vascular shutdown and the evoking of immunologic responses, there is also an element of direct cell kill that can reduce the neoplastic cell population by 2–3 logs.¹ This might be useful in a surgical context since it has been estimated that tumor cells are often left at surgical margins.²

It was initially believed that PDT killed cells via necrosis, and in 1991, Oleinick's group demonstrated that PDT could trigger the induction of apoptosis.³ Numerous subsequent studies documented the induction of apoptosis in a variety of cell types, by an array of different sensitizers and a variety of mechanisms. The cytotoxicity of PDT protocols, however, cannot be fully explained by the apoptotic response. Cells lacking procaspase-3⁴ or Bax⁵ die after PDT, but do not display the characteristics of necrotic or apoptotic cell death.

Autophagy was originally characterized as a survival response to nutrient deprivation and other forms of cellular stress,⁶ but studies within the past decade clearly indicate that the induction of autophagy can sometimes lead to cell death.^{7–13} Indeed, autophagic death is important during development,¹⁴ and appears to mediate the cytotoxicity of some anti-cancer agents.¹⁵ In this study, we assessed the possibility that PDT may induce autophagy, and that type II programmed cell death contributes to the cytotoxicity occurring in PDT protocols.

We employed a photosensitizer [9-capronyloxytetrakis (methoxyethyl) porphycene, or CPO], known to localize in the ER and catalyze Bcl-2 photodamage.¹⁶ Clonogenic assays were used to determine loss of viability by murine leukemia L1210 or DU145 prostatic tumor cells. Sixty min after irradiation of CPO-sensitized cells (LD₉₀ conditions), condensed chromatin was detected in approximately 50% of the cells by HO33342 labeling, whereas vacuoles were seen in only a few cells. When cells were incubated with the PI 3-kinase inhibitor wortmannin prior to irradiation, a greater percentage of the cells displayed apoptotic nuclei and none were vacuolated. PI 3-kinase activity is required for the development of autophagy.⁶ After 24 hr, in cultures treated with PDT alone, the apoptotic cells had mainly disappeared from the population and the survivors were swollen cells filled with vesicles. Electron microscopy demonstrated that these vesicles displayed the typical double-membrane structure associated with autophagy (Fig. 1). At this time point, >90% of the cell population was still impermeable to propidium iodide (PI).

Figure 1. Electron microscopy of a uranium/lead stained L1210 cell preparation demonstrating the presence of a double membrane in autophagic vesicles after photodynamic therapy.

After an additional 48 hrs the cell population exhibited a chaotic morphology and >90% PI permeability. In contrast, cells exhibited a much more normal morphology 72 hr after PDT when both wortmannin and the caspase 3/7 inhibitor zDEVD-fmk were present; in this case, no cells exhibited condensed chromatin and all were impermeable to PI.

Oleinick's group reported that PDT-induced cell death in the Bax-deficient DU145 prostate cell line⁵ and a Bax knock-out HCT116 cells line¹⁷ was neither necrotic nor apoptotic.⁵ We found that DU145 cells were killed by PDT protocols employing the ER photosensitizer CPO without development of apoptotic morphological features, loss of mitochondrial potential, formation of condensed nuclear chromatin or the activation of pro-caspases 3/7. Instead, CPO-sensitized DU145 cultures treated with an LD₉₀ PDT dose initially displayed the appearance of vacuolated cells with an unaffected mitochondrial membrane potential. The presence of PI 3-kinase inhibitors effectively suppressed this vacuolization of DU145 cells. These results suggest that cell death in this case may be due to autophagy.

Although autophagy was originally described as a cell survival response, numerous studies have also implicated it as a death pathway even when apoptosis is also occurring.¹³ One explanation for the development of autophagy after photodynamic therapy lies in the ability of CPO to cause photodamage to the protein Bcl-2,¹⁶ an effect also seen with another sensitizer that also targets the ER.¹⁸ In addition to pro-apoptotic effects, downregulation of Bcl-2 is associated with autophagy.^{19,20} Cells that received an LD₉₀ PDT dose in the presence of both zDEVD-fmk and wortmannin appeared to be morphologically normal 3 days later, consistent with a cytostatic effect when both apoptosis and autophagy are blocked.

Buytaert et al. also reported an autophagic response in HeLa cells after ER photosensitization with hypericin.²¹ This effect was associated with calcium fluxes and occurred only when apoptosis was prevented by Bax/Bak deletion. In the L1210 cell system, calcium fluxes are not a factor in initiation of autophagy after ER

photodamage,²² and both apoptosis and autophagy appear to be functional regardless of the Bax/Bak status.

Finally, in the context of photodynamic therapy, it is interesting to note that autophagy can result in MHC class II presentation of antigens derived from cytosolic proteins.^{23,24} The autophagic response to photodynamic therapy may therefore provide an explanation for the finding that treatment of tumor cells with low-dose PDT can yield anti-tumor vaccines.²⁵

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