Induction of Prosurvival Molecules During Treatment: Rethinking Therapy Options for Photodynamic Therapy

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Abstract
Photodynamic therapy (PDT) not only causes direct cytotoxicity to malignant cells within a tumor but also appears to have both direct and indirect effects on nonmalignant components of the tumor microenvironment. A host of preclinical studies have been performed to document how PDT modulates the tumor microenvironment. This article explores the role of cellular components such as the hypoxia-inducible factor 1α, vascular endothelial growth factor, cyclooxygenase-2, matrix metalloproteinases, the antiapoptotic protein survivin, and 17-AAG (an inhibitor of heat shock proteins), with the hope that combined modality regimens targeting these processes may improve PDT tumor responsiveness. (JNCCN 2012;10[Suppl 2]:S35–S39)

“We may want to rethink using photodynamic therapy [PDT] not as a single modality but in a multimodality approach,” declared Charles J. Gomer, PhD, Professor, Cancer Biology and Pediatrics at The Saban Research Institute, Children’s Hospital. Los Angeles, California. In an attempt to understand the suboptimal results with ocular PDT for retinoblastoma, Dr. Gomer and colleagues have focused their research on the tumor microenvironment. “In essence, what is happening is that there is a very sophisticated and complex microenvironmental effect of PDT in addition to a direct tumor cell effect,” he explained. Preclinical studies have centered on how PDT modulates this tumor microenvironment, focusing on an array of cellular components that may lead to tumor recurrence, many of which are briefly discussed in this article. One primary goal in identifying these cellular processes is to fashion therapeutic approaches to target them, thereby potentially enhancing PDT-mediated cancer treatment.

Learning From the Suboptimal Results of Ocular PDT for Retinoblastoma
“Many treatments [of retinoblastoma] are fairly successful, but there are recurrences,” admitted Dr. Gomer. With the late side effects from chemotherapy and radiation therapy, investigators have sought other alternatives. “Lesions on the retina and not floating in the vitreous seas are amenable to PDT,” he stated. Depending on the size, location, and stage of the tumor, treatment options include enucleation, external-beam radiotherapy, localized plaque radiotherapy, photoagulation, and laser thermal therapy.

Preclinical studies examined the cytotoxicity induced by hematoporphyrin derivative photoradiation in both normal and experimental tumor tissue in the eye.1,2 “We looked at distribution and long-term effects of PDT in the eye and thought we were ready to do clinical work using PDT for retinoblastoma and a variety of adult ocular melanomas,” revealed Dr. Gomer. The initial clinical results with ocular PDT were disappointing, he admitted. With low doses of PDT, no response was seen (< 15% reduction in tumor size). With some lesions, a complete response was seen for 2 to 3 months, explained Dr. Gomer, but in all cases, the tumors re-
grew over time. “We treated a variety of melanomas, some pigmented, and we could not get an effective response with PDT,” he stated.

One of the initial reasons why ocular PDT was not more successful in treating retinoblastoma was the actual delivery systems. “We could localize the light beam to the lesion, but it was certainly not an ideal system,” revealed Dr. Gomer. Now much more sophisticated and exact ocular delivery systems are available. Although the delivery systems were an issue, perhaps the main reason for the suboptimal results was related to the tumor microenvironment.

Targeting the Tumor Microenvironment: Focus on Select Prosurvival Molecules

A better understanding of the cellular factors that seem to modulate PDT responsiveness may be the key to future combined treatment alternatives with PDT for ocular tumors (Figure 1). In essence, PDT hits the tumor microenvironment, which induces angiogenesis, cell proliferation, and cell invasion, thereby leading to tumor recurrence, simplified Dr. Gomer. Therefore, “If we can target that microenvironment and those molecules that may be involved in PDT treatment recurrence, we may get enhanced effectiveness of the therapy,” Dr. Gomer discussed many of the angiogenic and prosurvival molecules being explored in preclinical studies, and some of the more promising ones that are being targeted to enhance the therapeutic effectiveness of PDT.

Hypoxia-Inducible Factor-1α

Characterized by sustained angiogenesis, hypoxia-inducible factor-1α (HIF-1α) is a client protein linked to cancer. Studies in a xenograft model of Kaposi sarcoma and in mouse mammary carcinoma cells and tumors have shown that PDT induces the increased expression of HIF-1α. In whatever tumor model we are looking at, within 1 to 2 hours, PDT induced significant expression of that transcription factor,” reported Dr. Gomer. Induction of hypoxia may play a role, but it can be induced by a variety of pathways, including an oxidative stress pathway, he added. Moreover, the dose rate also appears to affect the expression of this transcription factor: “The higher dose rate induced larger amounts of HIF-1α,” Dr. Gomer revealed.

Vascular Endothelial Growth Factor

In addition to HIF-1α, expression of vascular endothelial growth factor (VEGF) is induced by PDT. Porfimer sodium-mediated PDT was shown to be a strong activator of VEGF within the tumor microenvironment using a mouse tumor model. “In this model, it looks like the VEGF is coming primarily from the tumor cells themselves,” revealed Dr. Gomer. In another study, significant overexpression of Kaposi sarcoma cell–derived human VEGF, and to a lesser extent overexpression of host cell–derived mouse VEGF, was detected within treated tumors. Furthermore, as with HIF-1α, the dose rate played role: a low dose rate induced less VEGF than a high dose rate based on this mouse model, he added.

Through blocking the effects of VEGF and HIF-1α, it theoretically may be possible to improve results with PDT, hypothesized Dr. Gomer. He shared his results with bevacizumab (Avastin), a VEGF inhibitor, in combination with PDT in a Kaposi sarcoma tumor model. Gomer and Ferrario found that combining PDT with bevacizumab resulted in a significant increase in the long-term responsiveness of treated Kaposi sarcoma tumors compared to control.
with individual treatments (Figure 2). “Ninety days after treatment, there was about a 50% cure rate with the combination treatment, compared with a 20% to 25% cure rate with PDT alone,” he added.

**Bone Marrow–Derived Cells**
CD45+ monocytes can colonize perivascular sites of tumors and promote angiogenesis and tumor proliferation by paracrine mechanisms, whereas CD45- endothelial progenitor cells (CEPs) can merge into growing blood vessels, differentiating into mature endothelial cells, according to Dr. Gomer. PDT appears to increase circulating endothelial cells (CECs) and CEPs in peripheral blood. “The CECs are coming from the lesions themselves and being sloughed off; the CEPs are coming from the bone marrow and being liberated from there,” Dr. Gomer explained. “At a subcurative dose, 24 hours later, PDT induced both CECs (which could be a biomarker of treatment response) and CEPs (which could play a role in an enhanced angiogenic response).”

**Stromal Cell–Derived Factor 1α**
The small cytokine stromal cell–derived factor 1α (SDF-1α) is also induced by PDT. Based on a mammary carcinoma tumor model, there is overexpression of SDF-1α by PDT within that tumor,” reported Dr. Gomer. In addition, “SDF-1α may play a role in the responsiveness, or lack of responsiveness in some cases, of PDT.” The role of this cytokine in tumor responsiveness to PDT is still under investigation.

**Cyclooxygenase-2**
The isoenzyme cyclooxygenase-2 (COX-2), which catalyzes prostaglandin synthesis, seems to be involved with tumor growth, metastasis, and angiogenesis, stated Dr. Gomer. It has been determined that PDT induces increased expression of COX-2–derived prostaglandins (prostaglandin E2 [PGE2]) using tumor mouse models. “Not only was the enzyme expressed, it was a functional enzyme,” clarified Dr. Gomer.

Ferrario et al evaluated the role of COX-2 inhibition in the enhancement of PDT; and found that the COX-2 inhibitor celecoxib blocked PDT-induced expression of PGE2, improving the long-term tumoricidal activity without increasing normal tissue photosensitization. “With the combination of PDT and celecoxib, we saw the complete attenuation of PGE2 within the cell culture, which could explain why we saw recurrence in our retinoblastoma results,” said Dr. Gomer. Their results suggest that celecoxib-mediated enhancement of PDT may involve both COX-2–dependent and COX-2–independent mechanisms. However, side effects such as cardiovascular injury with the use of coxib-based COX-2 inhibitors may limit the clinical application of this class of compounds.

**Matrix Metalloproteinases**
Matrix metalloproteinases (MMPs) belong to a family of zinc-containing enzymes. These breakdown extracellular matrix proteins are involved in tumor cell migration, metastasis, and angiogenesis, declared Dr. Gomer. Preclinical studies have shown that porfirimer sodium–mediated PDT is a strong activator of MMPs. In particular, MMP-2 and MMP-9 may be overactivated by PDT.7 “PDT activates MMP-9 quite readily,” stated Dr. Gomer. In terms of cell type specificity for PDT-induced MMP-9, within the tumor cells there is a minimal effect on the expression of MMP-9; however, in the endothelial cells there is activation and increased expression of MMP-9, and in macrophage cell lines there is a high sensitivity to PDT and good activation and expression of MMP-9, according to Dr. Gomer.

Ferrario et al also evaluated the efficacy of a synthetic MMP inhibitor, prinomastat, to enhance...
tumoricidal activity after PDT in a mouse mammary tumor model. They found that PDT plus prinomastat may improve tumor response. “We saw a modest increase in the effectiveness of blocking the MMP expression and function,” revealed Dr. Gomer. Therefore, preliminary data suggest that using MMP inhibitors may potentially enhance clinical PDT.\(^5,10\) (Although prinomastat was in clinical trials, Dr. Gomer indicates that it is not being studied further at this time.)

**Survivin and 17-Allylamin-17-Demethoxygeldanamycin**

Heat shock proteins are overexpressed in a wide range of human cancers and are implicated in tumor cell proliferation, differentiation, and invasion.\(^3\) Dr. Gomer focused attention on the molecule survivin and 17-allylamin-17-demethoxygeldanamycin (17-AAG).

Increasing interest has centered on survivin, a member of the inhibitor of apoptosis family. Its role in response to PDT is being studied, but its role in regard to ionizing radiation and some chemotherapeutic agents is also under investigation, explained Dr. Gomer. PDT has been observed to induce the expression and phosphorylation of survivin in murine and human cancer cells and tumors.\(^10\) Although survivin is highly expressed in most human tumors and fetal tissue, it seems to be absent in terminally differentiated cells. This protein also inhibits caspase-9 and may be associated with resistance to chemotherapy and radiotherapy.\(^10\) Finally, survivin seems to localize to the mitotic spindle through interacting with tubulin during mitosis, perhaps playing a contributing role in regulating mitosis.

An analogue of geldanamycin, 17-AAG binds to heat shock protein 90 (Hsp-90) and alters its function.\(^10\) It also leads to misfolding of client proteins, ubiquitination, and proteasome degradation.\(^10\) Dr. Gomer and colleagues attempted to target Hsp-90 with 17-AAG to enhance the therapeutic effectiveness of PDT.\(^5,10\) They found that tumor-bearing mice treated with PDT and 17-AAG had improved long-term tumoricidal responses compared with those treated with PDT alone (Figure 3).\(^5\) In PDT-treated cells, 17-AAG attenuated survivin expression; “after combining PDT and 17-AAG, survivin returned to minimal levels,” revealed Dr. Gomer.

Investigators of these studies conclude that targeting survivin and perhaps other Hsp-90 client proteins may improve responsiveness to PDT.\(^5,10\)

“We are now looking at more specific inhibitors of survivin, such as YM155, which is being studied in phase II clinical trials in advanced cancers,” Dr. Gomer added. YM155 is a novel selective survivin suppressant, which may inhibit tumor spread and ultimately prolong survival.

**Conclusions**

Preclinical studies have helped to shed more light on the role of the complex tumor microenvironment in the growth of tumor cells and the responsiveness to treatments such as PDT. Clarifying these cellular components and their molecular pathways may lead to the development of more effective targeted combinations of therapeutic options for treating ocular tumors and other malignancies. “By combining PDT with targeted inhibitors, those in clinical trials as well as those already accepted clinically, we could affect a decrease in the tumor recurrence rate,” concluded Dr. Gomer.

**References**


