Photodynamic Therapy Mediates Immediate Loss of Cellular Responsiveness to Cytokines and Growth Factors

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ABSTRACT

Photodynamic therapy (PDT) is a minimally invasive procedure with increasing promise in treatment of malignant and nonmalignant diseases. Most PDT studies have focused on issues of how to enhance the phototoxic reaction leading to apoptosis and/or necrosis of targeted tumor cells. However, the reactions of surviving cancer cells, as well as normal host cells, are important elements that contribute to the outcome. Little is known about how these cells at sites of treatment react to inflammatory cytokines and growth factors that are elicited by PDT. To answer this question, we treated several epithelial cancer cell lines and normal epithelial and stromal cells with membrane- and mitochondria-damaging PDT. At different time points after PDT, cells were stimulated with interleukin 6 class cytokines or epidermal growth factor (EGF). Cellular responsiveness was determined by the activation of signaling proteins. We found that within the time period of PDT reaction, both normal and malignant cells lost their responsiveness to the cytokines and growth factor in a PDT dose-dependent manner. Photosensitizers targeted to the plasma membrane or mitochondria had similar effects. The recovery of responsiveness required 48–72 h and was accompanied by resumption of cell proliferation. Although the loss of EGF response could be explained by the immediate degradation of EGF receptor, the loss of cytokine responsiveness was only partly correlated with a reduction in cytokine receptor proteins. A PDT-mediated reduction of Janus protein tyrosine kinase-1 was also observed in HeLa cells. Our results demonstrate that PDT alters the regulatory capability of normal and tumor cells by lowering the responsiveness to factors that are known to assist in tissue repair and immune response. This effect of PDT has to be considered when predicting outcome of PDT.

INTRODUCTION

PDT is an experimental treatment that shows great promise in treating neoplastic and nonneoplastic diseases (1, 2). PDT requires a photosensitizer, light, and molecular oxygen (3). After excitation with visible light, singlet oxygen and other ROS are generated that cause oxidative damage to cellular components (3–5). The subcellular localization of the photosensitizer is of particular importance because it determines the sites of the primary oxidative damage. Close proximity of photosensitizer and the target is necessary because singlet oxygen can diffuse <10 nm during its lifetime.

PDT initiates a stress response that triggers either cellular repair and recovery or cell death by apoptosis and/or necrosis (6). Although PDT is designed to cause a cytotoxic reaction in tumor tissue, a post-PDT response involving inflammatory, innate, and adaptive immune reactions is envisioned to assist in successful eradication of residual surviving tumor cells (7, 8). Because tumor cells, similar to normal cells, are receptive to inflammatory mediators, a growth-modulating effect of these factors in tumor cells is expected (9–11).

An inhibition of EGF response through down-regulation of EGFR-tyrosine kinase has been noted in PDT-treated cells in vitro and in vivo (12). PDT has also been shown to alter the expression of cytokines at sites of treatment, including TNF (13), IL-10 (14), and, in particular, IL-6 (14–16). However, the influence of PDT on the cellular responsiveness to IL-6 and related type cytokines is not known. Therefore, we decided to identify the biochemical changes that are induced by PDT and which determine the cytokine responsiveness of several normal and transformed cell types. We show, for the first time, the effective abrogation of cytokine signaling and lasting attenuation of EGFR expression and action.

MATERIALS AND METHODS

Cell Lines. The following cell lines were obtained from the American Type Culture Collection (Manassas, VA): hypopharyngeal carcinoma FaDu; cervical adencarcinoma HeLa; and hepatocellular carcinoma HepG2. Primary cultures of human pulmonary fibroblasts and epithelial cells were prepared from residual lung tissues derived from surgical pneumectomy specimens. Use of human lung tissue is approved by the Institute Review Board, and prior informed consent of the participating patients has been obtained. FaDu cells were grown in EMEM with 10% FCS, and all other cell types were maintained in DMEM. Cells were treated with 10% FCS at 37°C in an atmosphere with 100% humidity and 5% CO2.

PDT and Stress Treatments. For mitochondria-targeted PDT, different concentrations (0–5 μM) of ALA (Sigma, Saint Louis, MO) were added to medium containing 1% FCS for 4 h (4). Cells, generally at 85–95% confluence, were exposed from above to red (570–650 nm) light at 10 J/cm2 and fluence rate of 6.3 mW/cm2 (GTE Sylvania, Salem, MA). For PDT, primarily targeted to the plasma membrane, the cells were incubated for 1 h in medium containing 5 μg/ml Photofrin and then irradiated with 1 J/cm2 (4, 17, 18). Photocytotoxicity was determined by tetrazolium salt MTT assay 24–48 h after PDT. At different post-PDT time points, cells were treated for 15 min with serum-free medium containing 100 ng/ml human EGF (Collaborative Research, Inc., San Jose, CA), IL-6, LIF (Genetics Institute, Cambridge, MA), OSM (Immunex Corp., Seattle, WA), 10 μg/ml TNF-α (In vitrogen Corp., Carlsbad, CA), or 1 μg/ml PMA (Sigma). Alternative stress responses to PDT were elicited by exposing cells for 2 h to 10 μM As2O3 (Cell Therapeutics, Inc., Seattle, WA), 10 μM methylselenenac acid (provided by Dr. Clement Ip, Roswell Park Cancer Institute), or UVA (303/307 nm). Cells were exposed with radioimmunoprecipitation assay lysis buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% Brij96, 0.25% sodium deoxycholate, 1 mM NaF, 1 mM sodium orthovanadate, 1 μg/ml leupeptin and aprotinin, 1 mM phenylmethylsulfonyl fluoride, and 10% glycerol).

Western Blot Analysis. Aliquots of cell extracts containing 10 or 20 μg of protein were electrophoresed on 6–10% polyacrylamide gels. The proteins were transferred to Protean membranes (Schleicher & Schuell, Keene, NH) and reacted with antibodies to p44/42 ERK, JNK, p38/HOG, EGFR (Cell

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4 The abbreviations used are: PDT, photodynamic therapy; ALA, aminolevulinic acid; As2O3, arsenic trioxide; ATF-2, activating transcription factor 2; EGF, epidermal growth factor; EGFR, EGF receptor; ERK, extracellular signal-regulated kinase; JAK, Janus protein tyrosine kinase; JNK, c-Jun NH2-terminal kinase; LIF, leukemia inhibitory factor; LIFR, leukemia inhibitory factor receptor; IL, interleukin; OSM, oncostatin M; OSMRβ, OSM receptor β; TNF, tumor necrosis factor; MAPK, mitogen-activated protein kinase; MCL-1, myeloid cell factor-1; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PMA, phorbol-12-myristate 13 acetate; ROS, reactive oxygen species; STAT, signal transducers and activators of transcription.
Signaling Technology, Inc., Beverly, MA), OSMRβ (Immunex Corp., Seattle, WA), LIFR-α, human gp130, JAK1, Cdc6 (Santa Cruz Biotechnology, Santa Cruz, CA), STAT3, or the phospho-specific form for ERK, JNK, p38, or STAT3 (Cell Signaling Technology, Inc., Beverly, MA). Appropriate peroxidase-conjugated secondary antibodies (ICN Biomedicals, Aurora, OH) were used in PBS containing 0.1% Tween 20, 5% milk. Immune complexes were visualized by enhanced chemiluminescence reaction (Amersham Biosciences). In each experimental series, X-ray films were exposed for different lengths of time to obtain images with quantitative signals in the linear range of densitometry. Equal loading of samples was verified by probing with antibodies against total STAT3 or by Ponceau staining of the proteins immediately after transfer to the Protein membrane.

To determine several proteins in the same extract, multiple electrophoretic separations of aliquots from the lysates were carried out. Reusing membrane was avoided because of the difficulties of removing certain immunoglobulins from the membrane. Quantifications of immunoblot signals were carried out by digital scanning of the X-ray films followed by analysis with the ImageQuant program (Molecular Dynamics/Amersham, Piscataway, NJ).

RESULTS

PDT Activates Immediate Signaling by the Stress-activated Protein Kinase Pathways and Causes Degradation of MCL-1. ALA and Photofrin-PDT are potent inducers of apoptosis. However, the kinetics and extent of apoptosis differ among cell lines and generally is dependent on the type and dose of photosensitizer and light (4, 6). We found by staining with Hoechst 333421 and propidium iodide that FaDu cells showed signs of apoptosis and necrosis 5 h after low-dose ALA-PDT (0.1–0.3 mM ALA) and primarily of necrosis at doses higher than 0.4 mM ALA or with Photofrin-PDT (Fig. 1A). ALA dose-dependent PDT reaction of FaDu cells indicated that cell survival, as defined by MTT assay after 48 h, was 55–35% when using 0.4–0.6 mM ALA at 10J/cm² (Fig. 1B).

Cells treated with increasing doses of ALA-PDT also were analyzed immediately after PDT for the activity of three key signaling proteins, STAT3, ERK, and JNK. The already low pretreatment levels of phosphotyrosine-STAT3 and phospho-ERK-1 and ERK-2 were maintained low or additionally reduced (Fig. 1C). As will be shown below (Fig. 2), PDT did not appreciably reduce the level of STAT3 or ERK protein. The cellular response to PDT was evident by an increase of phospho-JNK reaching a maximum by 0.6 mM ALA (Fig. 1C). The PDT dose-dependent changes in these signaling proteins were proportional to the change in cell viability (Fig. 1B).

To characterize cellular signaling in response to PDT, we chose conditions for each PDT type that yielded 35% viable cells 48 h after treatment. First, we assessed the kinetics of the initial signaling events elicited by ALA-PDT (Fig. 2). Within the 26-min period of PDT photoreaction, the stress MAPK pathway already was activated, resulting in phosphorylation of JNK and p38. This activation was maximal 5 min after PDT and then gradually declined. The activation of these kinases was temporally correlated with the enhanced serine phosphorylation of ATF-2, one of the known substrates of these kinases (19). Interestingly, the MCL-1 (20), an antiapoptotic member of the BCL-2 family and only BCL-2 member detectable by immunoblotting in FaDu cells, was lost from the PDT-treated cells with kinetics similar to the activation of the stress MAPKs. The level of STAT 3 and ERK1/2 in the same cells remained constant, indicating that PDT did not promote a general loss of those signaling proteins.

PDT Abolishes Signaling by Cytokines and Growth Factor Receptors. The responsiveness of FaDu cells to the IL-6-type cytokines and EGF was determined by short-term treatment with LIF, IL-6, OSM, or EGF. The immunodetectable phosphorylation of ERKs and STAT3 served as measure of the activity of the receptors (Figs. 3, A and B). EGF increased phosphorylated ERKs, and the IL-6 cyto-

Fig. 1. The PDT dose-dependent effects on signaling and survival of FaDu cells. A, FaDu cells were treated with the indicated ALA-PDT reaction (10 J/cm²). Five h later, the cultures were stained with propidium iodide and Hoechst 333421. Apoptosis is evident by the shrinkage of cells, formation of apoptotic bodies, and absence of propidium iodide staining. The necrotic cells are recognized by propidium iodide staining. B, in three independent experiments, the ALA-PDT dose-dependent cytotoxicity was determined by MTT assay 48 h after PDT reaction. Mean and SE values are reproduced. C, immediately after ALA-PDT reaction or the corresponding no light (dark) control, the FaDu cell cultures were extracted and analyzed for the immunodetectable level of the phosphorylated forms for STAT3, ERK1/2, and JNK.
kines stimulated tyrosine phosphorylation of STAT3, with OSM the most effective. The same treatments immediately after ALA-PDT did not yield any detectable signaling (Fig. 3, A and B, panels labeled “Light”). However, the cells showed the characteristic stress response, including the activation of JNK and a reduction of phosphorylated ERK and Akt1.

PDT using Photofrin targeted to the plasma membrane in FaDu cells produced essentially the same loss of cytokine responsiveness (Fig. 3B, left panels). This immediate attenuation of receptor signaling after ALA and Photofrin-PDT was also observed in other cell types, including normal pulmonary fibroblasts (Fig. 3C) and epithelial cells (data not shown) and cell lines such as HeLa (Fig. 5) and HepG2 hepatoma cells (data not shown). For fibroblasts, PDT with ALA and Photofrin (Fig. 3C) eliminated the response to OSM and EGF. Interestingly, PDT also suppressed the activation of ERK by PMA (Fig. 3C, right panel), suggesting a PDT-dependent impairment of protein kinase C signaling. In fibroblasts, as in FaDu cells, IL-6 cytokines, EGF, PMA, and even TNFα had a minor to nondetectable stimulatory effect on the JNK pathway, and treatments with these factors after PDT were unable to modify the level of PDT-activated JNK (Fig. 3, A and C).

To determine the cause for the seemingly concerted loss of signaling by IL-6 cytokine receptors, we considered first a PDT-mediated reduction of JAKs, which transduce signaling by those receptors (21).
The immunodetectable level of JAK1, the JAK isoform primarily mediating signaling by the common receptor subunit gp130 (22), was not altered in FaDu cells by ALA or Photofrin-PDT (Fig. 3B). Similarly, the levels of JAK2 and TYK2, albeit low in FaDu cells, were not appreciably reduced by PDT (data not shown). These results suggest that a reduction of JAKs was not responsible for the loss of cytokine responsiveness in PDT-treated FaDu cells.

**PDT Reduces the Level of Receptor Proteins.** On the basis of the finding that PDT reduced EGFR protein (12), we next considered a similar PDT effect on the receptor subunits for the IL-6 cytokines. FaDu cells and lung fibroblasts were treated with PDT and the receptor proteins were measured by immunoblotting. As expected, ALA-PDT (Fig. 4A) and Photofrin-PDT (data not shown) caused a >90% loss of EGFR within the time period of light treatment in both cell types. This reduction of EGFR paralleled the loss of EGFR signaling toward the ERK pathway (Fig. 3, A and C). In FaDu cells, the EGFR level decreased even further during a subsequent 1-h culture period. Moreover, in each cell type tested in this study, the EGFR protein from PDT-treated cells migrated on the SDS gel with a slower electrophoretic mobility.

ALA-PDT treatment of FaDu cells and fibroblasts induced a major reduction of the fully processed plasma membrane form of LIFRα (Fig. 4A, top band in the panel marked “LIFRα”). In contrast, no appreciable reduction of OSMRβ and gp130 occurred. This result suggests that the PDT-induced loss of LIFRα protein may account for the loss of LIF responsiveness. However, the loss of OSM responsiveness cannot be explained by a reduction of OSMRβ or gp130 proteins, suggesting a PDT-mediated impairment of the functionality of those receptor subunits.

The activation of JNK and the change of EGFR are sensitive markers for the immediate PDT response. We asked whether a cellular stress introduced by other pathways would cause similar changes. Therefore, we treated FaDu cells with UVA, As2O3, or methylseleninic acid under conditions that resulted in a 30–50% cell death within 24-h posttreatment period. These stress treatments were effective in activating JNK to a variable level, however, only UVA caused a decreased electrophoretic mobility and some loss of EGFR (Fig. 4B). Furthermore, the same treatments did not appreciably attenuate signaling by IL-6 cytokine receptors (data not shown). The finding that only UVA and PDT altered the electrophoretic mobility of EGFR suggests that this effect, at least in part, be mediated by 1O2 (23).

The PDT-dependent reduction of EGFR and LIFRα suggested an efficient degradation. Two possible mechanisms, degradation by the proteosomal or by the lysosomal pathway, were experimentally tested. However, neither inhibition of proteosomal degradation by MG132 (Fig. 4C) nor suppression of endosomal/lysosomal degradation by chloroquine (data not shown) during the PDT reaction could prevent the loss of EGFR. Ongoing studies evaluate the possible contribution of plasma membrane-associated metalloproteinases (24).

**PDT Response of HeLa Cells Indicates an Alternative Mechanism of Loss of Cytokine Receptor Signaling.** HeLa cells such as FaDu cells have a robust signaling in response to IL-6, LIF, OSM, and EGF. However, HeLa cells differ from FaDu and the other cell types we have analyzed by a substantially higher expression of the IL-6 cytokine receptor subunits and JAK1 and, thus, permitted a more accurate assessment of proteins after PDT. HeLa cells responded to ALA-PDT (Fig. 5) or Photofrin-PDT (data not shown) by a dose-dependent activation of JNK (Fig. 5B) and cell killing (Fig. 5A). When HeLa cells were treated with OSM immediately after ALA-PDT and analyzed for activated STAT3, a PDT dose-dependent loss of OSM responsiveness was observed (Fig. 5B). We also detected, as in FaDu cells (Fig. 3A), a loss of fully processed LIFRα and EGFR and only a minor reduction of OSMRβ (Fig. 5B). More interestingly, JAK1 was reduced in parallel to LIFRα and EGFR. These data suggest that in HeLa cells, the PDT-induced loss of JAK1 considerably contributes to the attenuation of OSMR signaling.

Because at low ALA doses, PDT is primarily targeted to the mitochondria, the oxidative reactions elicited by PDT must effectively reach the plasma membrane to account for the loss of receptor proteins. The question arose whether ALA PDT also enhances degradation of nuclear proteins. HeLa cells express appreciable levels of cell cycle-controlling proteins such as Cdc6 that is part of the origin of replication complex and is highly susceptible to stress-induced degradation (25). In the ALA-PDT-treated HeLa cells, the Cdc6 level was, however, <2-fold reduced (Fig. 5). This finding suggests that PDT-mediated signaling is biochemically distinct from that of other stress reactions, in particular, induced by DNA damage (26).

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**Fig. 4.** The effects of PDT on the level of receptor proteins.

A. FaDu cells and lung fibroblasts were treated with ALA-PDT. Immediately after PDT (FaDu and fibroblasts) or after 60-min culturing (only FaDu cells), the cultures were extracted, and the immune-detectable level of the receptor subunit proteins indicated on the left was determined. The level of STAT3 served as loading control. The top band of OSMRβ and LIFRα represents fully processed plasma membrane receptor protein, whereas the bottom band represents intracellular and incompletely modified receptor proteins. B. FaDu cells were exposed to the following stress reactions: 30 J/cm2 UVA; 10 μM As2O3; or 10 μM methylseleninic acid. The effects on the level of EGFR and phosphorylated JNK were determined by immunoblotting immediately after reaction. C. FaDu cells were treated with 0.6 mm ALA for 4 h. Some of the cultures were incubated with 25 μM MG132 for 30 min before and during light treatment. The level of EGFR immediately after PDT treatment was determined by immunoblotting.
Recovery of Cytokine Responsiveness in PDT-treated Cells Is a Slow Process. When FaDu or HeLa cells were treated with ALA or Photofrin-PDT at LD₈₀, only 1–15% of cells still viable at the 48-h post-PDT stage resumed proliferation as measurable by colony formation. The other portion of the viable cell population remained adherent to the culture substratum and retained metabolic activity but showed signs of growth-arrested cells. A large fraction of those nonproliferating cells represented G₂-arrested cells as evident from increased cell size and presence of micronuclei (27).

To be able to determine the kinetics by which PDT-treated cells regained cytokine responsiveness, we reduced the PDT dose. FaDu cultures were treated with a LD₄₀ ALA-PDT, and the viable cell population was monitored over a 3-day period for proliferation and responsiveness to OSM (Fig. 6). On the basis of microscopic evaluation, the first mitotic cells were noted 16 h after PDT, and a close to normal growth rate was reached by 72 h after PDT. PDT reduced EGFR protein to <1% of the pretreatment level as determined by densitometry of the immunoblot signals (Fig. 6A). A recovery to 20% level was measured in the 24 and 48 h cultures of surviving cells, and by 72 h, the level has reached the 80% value. The same cells treated with OSM for 15 min prior extraction indicated that immediately after PDT, no signaling toward STAT3 and ERK was detectable (Fig. 6B). STAT3 activation by OSM was restored to a 35% level by day 1, a 30% level by day 2, and a 70% level by day 3. Surprisingly, the recovery of OSM-mediated ERK activation compared with STAT3 activation was delayed. Only trace levels of phosphorylated ERK were noted in OSM-treated cells 24 and 48 h post-PDT. After 72 h, ERK activation was 25% of that of pretreated cells. Attenuated receptor expression and function in FaDu were observed in four independently performed ALA-PDT series.

Taken together, these data indicate that PDT reaction is not only effective in producing cytotoxic reactions that lead to cell death within 6–24 h but also has a lasting suppressive effects on the cytokine responsiveness of the surviving cells. The temporal pattern of altered responsiveness to growth factors and cytokines in both tumor and normal cells needs to be taken into account when predicting the outcome of therapeutic interventions after PDT.

DISCUSSION

We report the novel finding that PDT causes an immediate attenuation of signaling by receptors for inflammatory cytokines. This altered cytokine responsiveness is predicted to affect functions of both normal and tumor cells in the post-PDT tissue environment and may determine the treatment outcomes in patients undergoing PDT.

The relationship between inflammation and cancer has gained increasing attention. A number of studies has suggested that the inflammatory cells (28) and cytokines (29) found in untreated tumors are more likely to contribute to tumor growth, progression, and immunosuppression than to promote an effective host antitumor response. Moreover, cancer incidence and progression may be subject to functional polymorphisms of inflammatory cytokine genes and to altered expression of inflammatory cytokines (29). Treatment-induced
local inflammation is a major component of PDT (30). Therefore, it is essential to understand the processes that govern survival of normal and tumor cells and the effects of inflammatory mediators on these cells after PDT. This article provides new information related to signaling by PDT, loss of cytokine responsiveness, and recovery of signaling functions.

Initiation of PDT Signaling. PDT-initiated signaling can lead to cell death or alternatively can trigger stress responses leading to cell survival. Most studies on PDT signals have focused on those that lead to cell death (1–6). Depending on photosensitizer and light dose, PDT-induced generation of ROS and caused apoptosis and necrosis. Necrotic response, as observed in high-dose and plasma membrane-targeted PDT (Fig. 1A), is generally attributable to extensive immediate damage to cellular membranes and organelles, causing cessation of vital metabolic functions and loss of cellular integrity. PDT-initiated apoptosis involves the recruitment of programmed destruction mechanisms that are dominated by the caspase-dependent degradation of cellular components (3–6). Although not presented, preliminary characterization of PDT-treated FaDu cells indicated a modest level of proenzyme activation for caspases 3, 7, and 9 that was detectable a few hours after PDT and was temporally correlated with the degradation of poly(ADP-ribose) polymerase. The time course of caspase activation was clearly delayed relative to the activation of the stress pathway and loss of cytokine signaling.

Nonlethal PDT reactions studied in vitro are considered to represent the reactions that occur in the PDT-surviving population of tumor and normal tissue cells at treatment sites in patients. The pathways activated by nonlethal and lethal PDT may qualitatively be the same but differ in the magnitude and duration of their activation. The activation of the stress MAPK pathway is one of the most consistently observed immediate reactions to various PDT regimens in different cell types (23, 31–34). Depending upon its mode of activation, the stress MAPK pathway is considered to direct survival or cell death. Our studies demonstrated the characteristic activation of JNK and p38, but not ERK, with both ALA- and short incubation Photofrin-PDT. These findings (Figs. 2 and 3) are in agreement with those reported by others (23, 31, 32, 34). A contrasting observation has been made by Tong et al. (35) who found that Photofrin-PDT enhanced phosphorylation of ERKs in Li-Fraumeni syndrome cells. A potential explanation for this ERK regulation is the distinct subcellular localization of Photofrin in the cells that were incubated with the photosensitizer for extended lengths of time.

The exact role of JNK, p38 MAPK, and/or ERK in programmed cell death remains ambiguous. Blocking in HeLa cells the activation of JNK and p38 provided greater photosensitization with hypericin-PDT (33). This result was interpreted to mean that both the JNK and p38 protect cells from apoptosis. In contrast, inhibition of the p38 blocked Pc4 PDT-induced apoptosis in LS174Y-R cells and, to a lesser extent, in CHO cells (34), implying that p38 provides a pro-apoptotic signal. The difference in response may result from differences in cell lines, photosensitizer, or overall level of PDT damage. An alternative explanation is that the balance between JNK/p38 MAPK and ERK activity in stressed cells may determine the balance in the activation of cell survival (e.g., nuclear factor-κB pathway) or cell death responses (e.g., caspase pathway; Refs. 36, 37).

Downstream targets of activated JNK and p38 are transcription factors such as ATF-2, MAX, GADD153, and cAMP-responsive element binding protein (38–40). These factors will determine in PDT-surviving cells the expression of a broad range of genes, including IL-6 (16, 41). IL-6, in turn, is able to exert autocrine/paracrine action. Hence, the simultaneous, PDT-mediated reduction in the function of receptors for IL-6 and other cytokines prevents the execution of the predicted cytokine effects on cells in the treatment field.

Loss of EGFR and Cytokine Responsiveness. EGFR is overexpressed in a wide variety of solid human tumors and is considered to enhance cellular proliferation, motility, adhesion, invasion, and angiogenesis (42). As a consequence, inhibition of EGFR activity is being examined as an approach for the management of solid tumors (43). In contrast, signaling by the receptors for IL-6 cytokines generally is associated with controlling function of differentiated cells (21, 44, 45) but also with suppressing proliferation of various epithelial cells (46, 47). PDT with the lysozyme-located photosensitizer monol-asparyl chlorine 6 showed increased apoptosis in IL-6 gene-transfected murine Lewis lung carcinoma cell line (48). In contrast, Jee et al. (49) reported overexpression of IL-6 in ALA-PDT-treated human basal cell carcinoma cells increased the antiapoptotic activity.

The predicted functions of EGF and cytokines are critically dependent on the receptor status of the target cells. With the identification of a drastic loss of signaling through these receptors in PDT-treated cells, a reevaluation of previous studies needs to be considered in regard to the potential changes of cytokine responsiveness. Two basic mechanisms are considered to account for the loss of signaling: (a) degradation of receptor proteins or signal-transducing protein tyrosine kinases and (b) inactivation of signaling molecules, including a reduction of cellular ATP (6).

The PDT-induced loss of immunodetectable plasma membrane receptor, as found for EGFR (Figs. 3–5; Ref. 12) and LIFRα (Figs. 4 and 5), does not have the hallmarks of the processes associated with receptor turnover, i.e., endocytosis and endosomal/lysosomal degradation (50). The PDT effects on receptors are rapid and insensitive to inhibition of proteasomal and lysosomal enzymes. Contribution by caspases to receptor degradation appears unlikely because of the temporally much delayed activation of these enzymes in PDT-treated cells. The rapid receptor loss suggests a mechanism that is operative at the plasma membrane and is activated by ROS or its downstream effectors. The receptor loss may involve the action of cell surface protease such as of the Adam family of metalloproteinases (24), but the biochemistry of this removal mechanism remains to be defined.

Recovery of Cytokine Responsiveness in Post-PDT Cells. Loss of receptor function in PDT-treated cells, regardless whether these are normal or tumor cells, predict that those cells will not react to the cytokines generated by activated inflammatory and stromal cells. Moreover, because the local inflammatory reaction itself is in part orchestrated by the local production and action of cytokine, intercellular communication that depends on cytokine recognition and responses must be impaired at sites of PDT treatment. PDT has been shown to induce expression of IL-6 in HeLa cells (15) and FaDu cells (16) in vitro and in a BALB/c mouse tumors in vivo (14). We predict that execution of inflammatory reactions at PDT sites are altered not only by the PDT-mediated death of resident cytokine-producing cells such as macrophages, mast cells, and stromal cells but also by the slow recovery of cytokine responsiveness in surviving cell population.

In regard to tumor cell control at site of PDT treatment, the temporal pattern of inflammatory cytokines and the cytokine responsiveness of the tumor cells will determine whether the milieu created by PDT acts growth suppressive (e.g., through action of OSM; Ref. 46, 47) or growth-promoting (e.g., EGF/tumor growth factor α). The relative activation of the stress pathways, JNK, p38, and ERK, has been suggested to determine, in part, the decision for cell survival or apoptosis (36, 37). Hence, it will be of interest to identify whether the PDT-induced alteration of MAPK pathway activities will render recovering tumor cells more susceptible to or more protected against a subsequent stress.

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