# Chapter 2
# Mechanisms of Resistance to Photodynamic Therapy: An Update

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**Abstract** Photodynamic Therapy (PDT) involves the combination of light and photosensitizer (PS). Therefore, it is possible for cells to develop resistance based on the doses of PS used or the light dose. The data compiled by several authors make it clear that the degree of cell resistance to PDT is highly dependent on the PS used; however, no cellular characteristics have yet been identified as predictors of PDT resistance. The mechanisms by which the treated tissue becomes resistant to the PS share some similarities to those found in general drug resistance and radio-resistance, and they are mainly related to both the bioavailability of the PS and to the mechanisms of detoxification of the generated reactive oxygen species. Among the features related to PDT resistance are: the expression of p-glycoprotein and ABCG2 transporters, the abrogation of apoptosis and autophagy, the induction of antioxidant defences, the induction of HSPs changes in cytoskeleton and adhesion, the induction of cyclooxygenases, the production of nitric oxide and hypoxia; these are some but not all of the factors involved in the development of resistance. As a general rule, all the authors that reported resistance to PDT have attributed this phenomenon to several factors acting in concert. In this chapter, we will review some of the most important aspects related to PDT resistance.

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V. Rapozzi, G. Jori (eds.), *Resistance to Photodynamic Therapy in Cancer,*
Resistance to Targeted Anti-Cancer Therapeutics 5, DOI 10.1007/978-3-319-12730-9_2

Several approaches to reversing of PDT resistance have been developed. Among them, the use of PS linked macromolecules, which are internalized into cells via endocytosis and accumulated in the endosome/lysosome compartments, whereby they could efficiently be disrupted after irradiation by the mechanisms of photochemical internalization.

**Keywords** Apoptosis · Chemoresistance · Cross-resistance · Mechanisms · MDR · PDT · Photodynamic therapy · Photosensitizer · Porphyrins · Resistance

## Abbreviations

| | |
|---|---|
| ALA | 5-aminolevulinic acid |
| BCRP | Breast cancer resistant protein |
| BPD-MA | Benzoporphyrin derivative monoacid ring A |
| CAM-DR | Cell adhesion-mediated drug resistance |
| CPO | [9-capronyloxy-tetrakis(methoxyethyl) porphycene] |
| DXR | Doxorubicin |
| ECM | Extracellular matrix |
| ER | Endoplasmic reticulum |
| ERK | Extracellular signal regulated kinases |
| EVs | Extracellular vesicles |
| FADD | Fas-associated via death domain |
| HIF1 | Hypoxia-inducible factor 1 |
| HO-1 | Heme oxygenase 1 |
| HSP | Heat shock proteins |
| HPPH | 2-(1-hexyloxethyl)-2-devinyl pyropheophorbide-a |
| iNOS | inducible NO synthase |
| L-NAME | NG-Nitro-L-arginine methyl ester |
| LOX | Lipooxigenase |
| MAPK | Mitogen-activated protein kinase |
| MDR | Multidrug resistance |
| MRP1 | Multidrug resistant associated protein 1 |
| m-THPC | 5,10,15,20-tetra(*meta*-hyroxyphenyl) chlorin |
| m-THPP | meso-tetra(3-hydroxyphenyl)porphyrin |
| NO | Nitric oxide |
| NPe6 | Mono-L-aspartyl chlorin e6 |
| Pa-PDT | Pheophorbide a-PDT |
| PCI | Photochemical internalisation |
| P-gp | P-glycoprotein |
| PDT | Photodynamic therapy |
| PpIX | Protoporphyrin IX |
| PS | Photosensitizer |

| | |
|---|---|
| PII | Photofrin II |
| SnET2 | Tin ethyl etiopurpurin I |
| SNP | Sodium nitroprusside |
| SOD | Superoxide dismutase |
| SPNO | Spermine NONOate |
| TOOKAD | Palladium-bacteriopheophorbide WST09 |
| TPPS2a | Disulfonated meso-tetraphenylporphine |
| VEGF | Vascular endothelial growth factor |

## Introduction

The technique known as Photodynamic Therapy (PDT) uses a photosensitizer (PS) that can be applied either systemically or topically in a confined area, which upon excitation with visible light [1, 2] will react in the presence of oxygen to form cytotoxic oxygen species [3]. PDT involves the combination of light and photosensitizer, therefore, it is possible for cells to develop different levels of resistance based on the doses of PS used and/or the light dosage during treatment [4–7]

The mechanisms by which the treated tissue becomes resistant to the PS share some similarities to those found in general drug resistance, e.g. (i) differential uptake rate or efflux (ii) altered intracellular trafficking of the drug (iii) decreased drug activation, and (iv) increased inactivation of drug.

After PDT has been initiated, reactive oxygen species are produced [3], and consequently, antioxidant defense mechanisms are triggered to counteract the damage [8, 9]. As a result, Heat Shock Proteins (HSP) as well as other HSP-assisting proteins are activated [10]. In a later stage, increased repair of proteins, membranes and DNA, together with the induction of stress response genes and consequent activation of several survival pathways are also triggered [11].

Over the years, cell lines with different degrees of resistance to PDT have been isolated. Understanding the underlying mechanisms of the PDT resistance will help to improve the efficacy of PDT and can generate new combination protocols, such as chemo- or radiotherapy and PDT, for a better and a more efficient patient treatment.

In 1991, Luna and Gomer [6], using a fibrosarcoma cell line (RIF-1) and Photofrin II (PII), generated PDT-resistant cells using two protocols: short exposure (damage to the plasma membrane) and long exposure (damage to the organelles and enzymes). The levels of resistance obtained with both protocols were stable and increased cell survival by 2.5–3.0 logs and 1.2–1.5 logs, respectively, at the highest light dose used.

Using the same parental RIF-1 cell line, Singh et al. [5] were able to isolate and characterize two resistant cell lines based on the long exposure protocol (8 cycles of PII-PDT), obtaining what they called RIF-8A. These cells showed a similar degree of resistance (2.0 logs in survival). When they implanted the cells into mice, they

found PDT resistance *in vivo*. Moreover, the cells isolated from those tumors after PDT treatment also showed some degree of resistance *in vitro*; however, the resistance index was lower, suggesting a role of additional factors derived from both the host and the microenvironment.

Based on the afore mentioned studies and those from Mayhew et al. [4], it is clear that the degree of cellular PDT resistance was highly dependent on the PS used, but no cellular characteristics had been identified as predictors of PDT resistance in the generated clones. It is believed, however, that the chemical structure of the PS used is a key factor in the development of resistance, as the structure determines the intracellular accumulation of the PS [12–15].

In recent years, 5-aminolevulinic acid (ALA)-mediated PDT has become one of the most promising leads in PDT. ALA is the pro-drug of the PS Protoporphyrin IX (PpIX). Using ALA-PDT, we generated two resistant clones from a murine mammary adenocarcinoma cell line. The clones exhibited 6.7- and 4.2-folds increase in resistance, respectively, compared to the parental cell line. On the contrary, no evidence of PDT resistance was found in the response of human glioma spheroids to repetitive ALA-PDT [16] treatment, suggesting that resistance to PDT comprises a broad number of aspects, and not all cell types and cell models develop resistance to the same PS.

We have previously reviewed the main features of PDT resistant cells, and we compared them with features commonly found in chemoresistant cells. We found that many mechanisms of resistance to PDT are shared with chemotherapy and radiotherapy [17]. Contributing to PDT resistance are (1) the expression of p-glycoprotein and ABCG2 transporters (2) induction of early response genes and signal transduction pathways (3) abrogation of apoptosis and autophagy (4) induction of antioxidant defenses (5) HSPs induction (6) changes in cytoskeleton and adhesion (7) induction of cyclooxygenases (8) production of nitric oxide (9) survivin expression (10) and hypoxia. These are some, but not all, of the factors involved in the development of resistance. As a general rule, all the authors that have reported resistance to PDT have attributed this phenomenon to several factors acting in concert. In this chapter we will review some of the most important aspects related to PDT resistance.

## Photosensitizer Uptake and Efflux in Resistant Cells

Results of recent studies suggest that PDT efficacy and/or resistance may depend on the uptake of PS [18] and alterations in the expression and function of key molecules involved in PS transport; these may be related to the emergence of resistance. PDT is also dependent on the cellular localization of the PII during treatment, and a key role has been given to the mitochondria. RIF-1 derivative resistant cells accumulated either slightly higher amounts of PS per cell compared to the parental cells with a lower amount of PS on a per mg protein basis [6] or similar amounts of PS but weaker co-localization with markers of the inner mitochondrial membrane

in the resistant variants. These findings suggesting that the inner mitochondrial membrane is a significant PII binding site and may be related to the mechanism of resistance [5, 19].

Our group also found that the amount of porphyrins accumulated per cell in ALA-PDT-resistant cells was similar to the parental cell line [7], but that the same amount was half of that found in the parental cell line protein content when expressed in a per mg basis. This means there is less available porphyrin to target the same amount of proteins [20]. If the amount of porphyrins and not the cell target molecules is the limiting factor in PDT damage, this feature can also induce resistance.

We have also reported alterations in the heme pathway that leads to a higher amount of hydrophilic porphyrins and lower amounts of PpIX in these ALA-PDT resistant cells. Since hydrophilic porphyrins such as coproporphyrin and uroporphyrin are known to have low efficacy as PSs, these factors could partly account for the development of resistance [21–23].

We have reported an observation of particular importance in ALA-PDT-based treatment—our resistance clones exhibit an increased number of mitochondria per cell. Since these organelles are responsible for the last step of ALA conversion into PpIX, it leaves no doubt about the key role of the mitochondria in PDT treatment [7]. In addition, PDT induces several forms of damage in the mitochondria, such as the inactivation of enzymes, uncoupling oxidative phosphorylation and the generation of toxic species, rapidly leading to apoptosis [24–29].

Sharkely et al. [30] also described the changes in the mitochondria found in their resistant clones. The organelles were smaller and more electron dense, with higher cristae density compared to the parental RIF-1 cell line. The total mitochondria area per cell in the resistant line was double that of the parental cell line, and the ATP content and succinate dehydrogenase activity were higher. However, the oxygen consumption rates were similar, suggesting an altered energy metabolism. On the other hand, the RIF-8A resistant cell line had a decreased mitochondrial potential.

In 1997, it was suggested that in the early stages of damage, the mitochondria protect themselves from oxidative stress by downregulating several mRNA and rRNA-encoding gene products [31]. Shen et al. found a reduction of the mitochondrial 16S rRNA and NADH dehydrogenase subunit 4 in the PDT-resistant variants of HT29 human colon adenocarcinoma [32]. While Singh et al. [33] found significant resistance to PDT in cell lines lacking mitochondrial DNA, resistance to alkylating agents or γ-irradiation, however, was not found; even though the mitochondrial function has been closely related to cell death by apoptosis [34], the resistance in the isolated lines was not due to changes in this mechanism.

In our ALA-PDT-resistant lines we found higher protein content, an increased number of mitochondria and a higher oxygen consumption rate [7]. However, when we normalized per ng protein content, the number of mitochondria was similar in both the resistant and the parental cell lines. Surprisingly, PpIX synthesis, which takes places in the mitochondria, was not increased in the resistant cells, suggesting some altered functions in these organelles.

## The Role of P-glycoprotein in the Efflux of PS

It is not yet completely understood what causes the multidrug resistance (MDR) phenotype, and the lack of understanding remains a major problem in oncological treatments. The p-glycoprotein (P-gp or ABCB1) is encoded by the *MDR1* gene, and it is one of the ATP-binding cassette (ABC) drug transporters of the drug efflux pump class for a broad number of antineoplastics. It is also one of the main molecules involved in the development of MDR.

With respect to PDT, no overexpression of P-gp in RIF cells was found after multiple PDT treatments [5, 6]. In addition, similar doxorubicin (DXR) uptake was found when comparing RIF-8A to its parental cell line, and no cross-resistance to DXR was observed [5, 35]. However, cross-resistance to cisplatin treatment was observed [5, 35]. Additionally, our group found that the ALA-PDT resistant clones are not resistant to cisplatin, DXR, 5-fluorouracil, mitomycin C or methotrexate treatments.

PDT resistance in chemoresistant cell lines is also variable. The Chinese ovary hamster CHO-MDR line exhibited cross-resistance to PII-PDT, and this behaviour was correlated with a lower PII accumulation [5]. Similarly, cross-resistance to PDT was found in P388/ADR murine leukemia cells resistant to DXR, utilizing a cationic chlorin PS, and the impaired PS accumulation suggested a correlation between the resistance and the increased P-gp efflux pump activity [36]. However, other authors reported that the same murine leukemia P388/ADR cells were not cross-resistant to mesoporphyrin-PDT, suggesting the impairment is more related to the PS structure and its affinity for the P-gp [37].

The relationship between the MDR cell phenotype and the ALA-PDT response is not clear. Tsai et al. [38] found that MCF-7/DXR cells accumulated lower levels of PpIX from ALA, as compared to the parental MCF-7. However, the effect of ALA-PDT in MCF-7/DXR cells was less effective than in MCF-7 cells even when they showed similar amounts of PpIX. These results suggest that the resistant cells might possess intrinsic mechanisms that render them less sensitive to ALA-PDT, and their resistance is not related to the MDR efflux of PpIX.

Intracellular levels of ALA did not increase substantially upon incubation of the MCF-7 TX200 cells transfected with a P-gp inhibitor [39]. Similarly, Li et al. [40] showed that the P-gp inhibitor verapamil did not induce changes in PpIX levels in MDR-resistant leukemia cells exposed to ALA. More recently, Chu et al. [41] employing ALA derivatives, showed that the human uterine sarcoma cells MES-SA-Dx5 overexpressing P-gp exhibited reduced intracellular levels of PpIX derived from hexyl-ALA to a limited degree, and this mechanism could be reversed by using verapamil. Hexyl-ALA-PDT induced a decrease in MDR1 mRNA levels in MES-SA-Dx5 cells (resistant to DXR) together with a concomitant decreased expression of P-gp [41]. Similarly, pheophorbide-PDT of the multidrug-resistant HepG2 cells induced c-Jun N-terminal Kinase activation, leading to a down-regulation of P-gp [42].

It was also suggested that the intracellular localization of the PS has some influence on the MDR phenotype. Selbo et al. [43] found the MES-SA-Dx5 cells to be more resistant to PDT with disulfonated meso-tetraphenylporphine (TPPS2a) via a process not mediated by the P-gp mechanism. Their findings suggest the influence of different endocytic vesicle localization of the PS and that lysosomal targeting by PDT induces a stronger cytotoxic effect than PDT targeting the endosomes. According to Chu et al. [41] and Tang et al. [42], this finding may be related to an indirect down-regulation of MDR or a mechanism different from drug efflux.

Neither chlorin e6 accumulation nor efflux was modified in MCF-7/DXR overexpressing P-gp as compared to the parental line, but its subcellular distribution was different. In addition, a P-gp inhibitor restored the distribution of the PS and restored the response to chlorin e6-PDT [44].

In conclusion, it appears that MDR confers a degree of PDT resistance in some cases, and PDT resistance is strongly dependent on the structure of the PS and its affinity for the P-gp, as well as its intracellular distribution. However, no rules have yet been found to determine cross resistance.

## *Reversal of MDR by PDT*

Sometimes, the MDR phenotype can be reversed by employing classic PDT. MCF-7 mammary carcinoma cells overexpressing P-gp or multidrug resistant-associated protein exhibited similar accumulation of chlorins, porphyrin-based PS and pheophorbides compared to the parental line [39], and the MCF-7/DXR, DXR resistant cells, were even more sensitive to meta-tetra(hydroxyphenyl)chlorin (m-THPC) [45].

Cheung et al. [46] showed that pheophorphide a-PDT was able to circumvent MDR in the P-gp overexpressing human uterine sarcoma MES-SA/Dx5 cells. Both intracellular accumulation of pheophorbide a and pheophorbide a-PDT-induced cell death were not abrogated by the MDR phenotype. Both activity and expression of MDR1 and P-gp were reduced by Pa-PDT treatment, and such reductions were attenuated by the ROS scavenger α-tocopherol. On the other hand, a higher light dose was needed to induce apoptosis in hexyl-ALA-PDT-treated MES-SA/Dx5 cells [47]. ALA–PDT also reversed the resistant phenotype of the cell lines LBR-D160 and LBR-V160 lines isolated from a murine T-cell lymphoid leukemia after increasing vincristine or DXR exposure [48].

In addition, alternatives to PDT have been developed to overcome chemoresistance. Palladium-bacteriopheophorbide WST09 (TOOKAD) is a new generation of hydrosoluble PS, which binds primarily to albumin and has a local effect in the vasculature, since it does not extravasate and remains constrained in the circulation. The effect of TOOKAD-PDT in HT29/MDR cells as well as derived xenografts shows that this therapy overcomes drug resistance [49].

Photochemical internalization (PCI) has been shown to help overcome chemoresistance in several MDR cell lines employing several PS and anticancer drugs [43,

50, 51]. PCI is a novel technology for the release of endocytosed macromolecules into the cytosol. Both PS and cytotoxic agents are localized in the same endocytic vesicle and internalized together into the target cell, where the PS is specifically localized to the vesicular membrane, and upon activation of PS by light, induces a release of the endocyosed macromolecules from their compartmentalization [52].

One mechanism for MDR is increased acidification of endocytic vesicles and increased cytosolic pH, so weak base chemotherapeutic agents, including DXR, are trapped in endocytic vesicles and exhibit a drug resistant phenotype. In MCF-7/ADR [50] cells that were preloaded with DXR, after PCI treatment the drug was released into the cytosol and entered cell nuclei, to the same extent than non-treated MCF-7 cells, thus, reversing the MDR phenotype by endo-lysosomal release of the drug.

Selbo et al. [43] also evaluated the reversal of resistance induced by PCI of macromolecules that were not targets of ABC drug pumps. Both MES-SA and MES-SA-Dx5 cell lines were equally sensitive to PCI of gelonin (ribosome-inactivating protein) using a low light dosage, even though the endocytosis rates were lower in the MDR cells. When higher light doses were employed MES-SA/Dx5 cells were more sensitive to PCI of gelonin than the parental cells. After adenoviral infection, PCI enhanced the fraction of transduced cells, in both cell lines, suggesting the potential use of PCI of macromolecular therapeutic agents that are not targets of P-gp as a strategy to eradicate MDR cancer cells. This PCI-mediated reversal of resistance circumvents ROS-induced photodamage in an apoptosis-independent manner [53].

In addition, some other approaches to reverse chemoresistance employing PDT utilize nanomedicinal tools to deliver the PS more efficiently and reduce the chances of being pumped out from the cytoplasm. In this regard, nanoparticle-based therapy integrative systems represent an emerging approach to overcome resistance. Ling et al. [54] recently reported the generation of tumor pH-sensitive magnetic nanogrenades (termed PMNs) composed of self-assembled iron oxide nanoparticles and pH-responsive ligands. These PMNs can readily target tumors via surface-charge switching triggered by the acidic tumor microenvironment, and are further disassembled into a highly active state in acidic subcellular compartments that “turns on” photodynamic therapeutic activity. These PMNs localized with high selectivity in mice tumors enabling pH-dependent PDT, and induced an increased therapeutic efficacy in drug-resistant tumors, showing a great potential for clinical applications.

## ABCG2-mediated Efflux of PS and Its Role in Cross Resistance

Another ABC transporter found to be capable of inducing resistance, in addition to P-gp, is a novel member of the G subfamily and has been described in the breast cancer cell line MCF7/AdrVp. This transporter was named the breast cancer resistance protein (BCRP), and is currently referred to as ABCG2 [55, 56]. Like all

members of the ABCG subfamily, ABCG2 is a half transporter, and it is believed to function as a mechanism of defense against toxins, regulating the traffic of toxic metabolites from and into the organism [57]. Among the drugs that can be effluxed by ABCG2 are mitoxantrone, camptothecin-derived and indolocarbazole topoisomerase I inhibitors, methotrexate, flavopiridol, and quinazoline ErbB1 inhibitors [58].

Several photosensitizers have been reported to be substrates of ABCG2 [59], including ALA-induced PpIX [13, 14], Pheophorbide a [13, 15], chlorin e6 [60], pyropheophorbide-a methyl ester [60], 2-(1-hexyloxethyl)-2-devinyl pyropheophorbide-a (HPPH) [12, 16], benzoporphyrin derivative monoacid ring A (BPD-MA) [12] and Hypericin [61]. On the contrary, meso-tetra(3-hydroxyphenyl)porphyrin (m-THPP), m-THPC [15, 60], hematoporphyrin IX [60] and HPPH-galactose [12, 16] TPPS(2a), di-sulfonated meso-tetraphenylchlorin (TPCS(2a)) and di-sulfonated aluminium phtalocyanine (AlPcS(2a)) are not substrates of ABCG2.

Using an ABCG2 knockout mouse model, Jonker et al. demonstrated that this transporter is able to efflux PpIX and protect cells from photodamage [62]. A correlation was also demonstrated between the expression of ABCG2 and resistance to PII-PDT [63]. Robey et al. [64] suggested that this transporter may be involved in the development of PDT resistance. In their studies, they compared the ability of ABCG2 to transport pheophorbide a and other PS with a similar structure. Bronchoalveolar carcinoma cells (NCI-H1650 MX50) overexpressing the transporter were found to have reduced intracellular accumulation of pyropheophorbide a methyl ester, chlorin e6 and PpIX, while the intracellular accumulation of hematoporphyrin IX, m-THPP, and m-THPC was not altered [39]. Afterwards, in line with the above mentioned findings, when human embryonic kidney HEK-293 cells were transfected with ABCG2, they exhibited resistance to PDT employing Chlorin e6, pheophorbide a, pyropheophorbide a methyl ester and ALA but not to m-THPC.

Multidrug resistance is suggested to be an important mechanism for the survival of cancer stem cells during therapy [65]. ABCG2 is a putative cancer stem cells marker and the molecular determinant of the Hoechst 33342 side population phenotype, which is detected as a distinct cell population with low blue and red fluorescence in flow cytometry dot blots [66]. Selbo et al. [59] demonstrated that strongly amphiphilic PSs used for PCI-based drug delivery are not substrates of ABCG2. They employed the breast carcinoma cell line MA11, with a Hoechst 33342 side population of $>50\%$ as an ABCG2 high expression model. Pheophorbide a and Hoechst 33342 were used as positive control substrates of ABCG2. ABCG2-inhibition by fumitremorgin C did neither induce an increased accumulation of three PCI-photosensitizers: TPPS(2a), TPCS(2a) and AlPcS(2a) nor enhanced the response of the cells to PDT. The same results were also obtained with TPPS2a in the malignant glioma cell line U87 having a SP of $\sim 0.1\%$. In contrast, both uptake and PDT-induced cytotoxicity was strongly enhanced for Pheophorbide a when combined with fumitremorgin C. On the other hand, EGFR + /ABCG2 + MA11 cells exposed to PCI employing the targeting toxin EGF-saporin were responsive to the treatment [59].

It has been suggested that the expression of ABCG2 transporter is decreased in a variety of cancers. Analyzing a set of normal and cancer paired tissues, Gupta et al.

[67] found that human samples from colorectal and cervical cancers had a down-regulation of ABCG2 mRNA levels.

Using a variety of human and mouse cell lines with a range in the expression of ABCG2 transporter, Liu et al. [68] studied the effect of tyrosine kinase inhibitors, which are able to block the ABCG2 function. The set of cell lines employed included: BCC-1 cells (basal cell carcinoma) and RIF-1 fibrosarcoma cells with high ABCG2 expression, Colo 26 (colon carcinoma) with moderate expression and FaDU (head and neck squamous cell carcinoma) with no expression of the transporter. In ABG2-expressing cells, an efficient efflux of HPPH, BPD-MA and PpIX was observed, while PII and HPPH-galactose were minimally transported. Treatment with a tyrosine kinase inhibitor increased the accumulation of the PS BPD-MA, PpIX, and HPPH in ABCG2 expressing cells, but not in the non-expressing cells. It also enhanced PDT efficacy both *in vitro* and *in vivo* in a RIF-1 tumor model. These results demonstrate on one hand that the use of ABCG2 inhibitors may increase the efficacy of clinical PDT, and on the other hand they show the importance of the PS structure and the affinity for the transporter in the development of resistance.

Although Robey et al. [39] suggested that Photofrin was not a ABCG2 transporter, Usuda et al. [69] using A431 cells overexpressing ABCG2 found they were resistant to PII-PDT but not to Mono-L-aspartyl chlorin e6 (NPe6)-PDT, which is similar in structure to m-THPC, and resistance to PII-PDT was reversed using a non-tyrosine kinase inhibitor of ABCG2 (Fumitremorgin C). In addition, in 81 centrally located early lung cancer lesions, > or = 10 mm in diameter, the expression of ABCG2 was inversely correlated with the outcome of PII-PDT while the transporter expression did not seem to affect the antitumor effect of NPe6-PDT.

Curiously, the modulation of ABC transporters by a PS has also been reported. Using HT-29 colon cancer cells treated with hypericin but no light, an increase in the activity of ABCG2 and the multidrug-resistant associated protein 1 (MRP1) transporters was reported [70].

Hypoxia regions are very commonly present in tumors and this oxygen depletion has been found to up-regulate the expression of ABCG2. It can also promote cell survival by decreasing the intracellular accumulation of porphyrins and heme [71]. Since it is well known that PDT requires the presence of oxygen to achieve its effect, hypoxia may inhibit PDT-depleting oxygen but also increase the ABCG2 transporter, which diminishes the intracellular levels of PS.

In summary, the resistance conferred by ABCG2 and P-gp transporters is directly related to the cell line and the PS employed, and in some cases, the resistant phenotype can be reversed by using specific transporter inhibitors.

Recently, Goler-Baron and Assaraf [72] identified a novel mechanism of MDR in which ABCG2-rich extracellular vesicles (EVs) form in between attached neighbor breast cancer cells and highly concentrate various chemotherapeutics in an ABCG2-dependent manner, thereby sequestering them away from their intracellular targets. These authors also showed that illumination of EVs that accumulated photosensitive cytotoxic drugs such as imidazoacridinones and topotecan resulted in intravesicular formation of ROS and severe damage to the membranes in the

vicinity of EVs leading to cell death and overcoming of resistance. Furthermore, consistent with the weak base nature of imidazoacridinones, MDR cells that are devoid of EVs but contained an increased number of lysosomes were efficiently killed via photodynamic lysosomal rupture. Combining targeted lysis of imidazoacridinone-loaded EVs and lysosomes elicited a synergistic cytotoxic effect resulting in the reversal of MDR. In contrast, topotecan, a substrate of ABCG2, accumulated exclusively in EVs of MDR cells but not in non-MDR breast cancer cells. This exclusive accumulation in EVs enhanced the selectivity of the cytotoxic effect exerted by photodynamic therapy to MDR cells without harming normal cells.

Taking advantage of the increased number of lysosomes in MDR cells, the same group [73] developed an imidazoacridinones-based PDT approach to eliminate MDR cancer cells. MDR cells overexpressing the MDR efflux transporters ABCG2, ABCB1 or ABCC1 and their normal counterparts were used: non-small-cell lung cancer A549 cells and their ABCG2-overexpressing A549/K1.5 subline, ovarian carcinoma 2008 cells and their stable MRP1 transfectant 2008/MRP1, and non-small lung cancer cell line SW1573 and its ABCB1-overexpressing subline SW1573/2R160. PDT resulted in 10- to 52-fold lower IC-50 values of various imidazoacridinones, thereby, restoring parental cell sensitivity. Finally, *in vivo* application of PDT-imidazoacridinone to human ovarian tumor xenografts in the chorioallantoic membrane model revealed selective destruction of tumors and their associated vasculature after two consecutive treatments.

## Apoptosis and Resistance to PDT and its Relationship with PS Structure

As a result of apoptosis induction by different stimuli, a series of signaling pathways are activated. As it has been previously addressed, PDT is known to up-regulate several signaling pathways, some of which on one hand may act as mediators or promoters of apoptosis as a result of the PDT treatment. On the other hand, some pathways are related to promote damage repair [74] and, therefore, could be linked to the development of resistance. In this regard, there are controversial evidences as some studies correlated the presence of apoptosis with the appearance of resistance, whereas in a few cases, altered apoptosis pathways have been found in cells resistant to PDT.

There have been different studies that linked the type of cell death and the PS subcellular localization [29]. Therapy can induce photodamage in the different organelles or in the plasma membrane. It has been demonstrated that anti-apoptotic proteins of the Bcl-2 family were targeted by PDT if the PS was localized in the endoplasmic reticulum (ER) or the mitochondria. On the other hand, when the plasma membrane was targeted by PDT, inhibition or delayed apoptosis and rescue responses were triggered [28, 75].

The subcellular localization of the PS varies according to its chemical nature and the molecular structure. Due to the hydrophobic nature of most PS they tend

to bind to membranes, e.g., ER, mitochondria, Golgi, lysosomes and plasma membrane [76]. Kessel et al. [77] evaluated the PDT responses to two structurally-related photosensitizing agents, using P388 murine leukemia cells. The PS tin etiopurpurin (SnET2) induced photodamage in lysosomes and mitochondria yielding a rapid apoptotic response. Using the analog tin octaethylpurpurin amidine (SnOPA), which targeted lysosomes, mitochondria and cell membranes yielded apoptotic evidence only after 24 h post PDT, suggesting photodamage to the membranes can delay or prevent an apoptotic response to PDT, thus resulting in resistance to PDT.

Along the same lines, Dellinger et al. [78] reported that PDT of cells exposed for a short period to high concentrations of PII exhibited an aborted form of apoptosis and attributed this to the leakage of cytoplasmic content through photodamaged membranes.

The Kessel group found that SnOPA and a monocationic porphyrin relocalized to the cytosol during irradiation, and this feature was correlated to delay or inhibition of apoptosis [75]. Employing m-THPC, SnET2 and 9-capronyloxy-tetrakis (methyoxyethyl) porphycene (CPO) in fluorescence localization studies, this group was also able to show that the PSs that theoretically targeted mitochondria, were also able to bind to a variety of intracellular membranes. These PSs generated damage through apoptosis, affected Bcl-2 levels, but did not affect the pro-apoptotic protein Bax. According to the accumulation site, the apoptotic response was induced soon after treatment with m-THPC and CPO, while the response was delayed when treated with SnET2 (associated with lysosomal photodamage) [79].

Utilizing positively charged porphyrins, Kessel et al. [80] showed that the position of the charge influenced the intracellular localization sites. The PS (5,10-di[4-(N-trimethylaminophenyl)-15,20-diphenylporphyrin) can penetrate the plasma membrane easily and target mitochondria, resulting in a rapid loss of membrane potential and triggering apoptosis. On the other hand, 5,15-di[4-(N-trimethylaminophenyl)-10,20-diphenylporphyrin with a different charge distribution targets lysosomes instead, resulting in extensive photodamage but with less effectiveness than the former pathway.

The same group using NPe6, which is an amphiphillic PS that targets the endosomal/lysosomal membranes, demonstrated that it is possible to induce indirect activation of apoptosis. Upon damage to these vesicle membranes, there is a leakage of enzymes that mediate the activation of the apoptotic lysosomal pathway involving the release of cathepsin B and cleavage of Bid which, in turn, can interact with the mitochondria leading to the release of cytochrome c and the activation of caspases -3 and -9 [81].

In addition to apoptosis, autophagy can also occur after PDT, especially when low light doses are applied [82]. This process acts as a pro-survival response due to recycling of damaged organelles, offering protection from photodamage [83, 84]. The role of authopagy in PDT resistance will be addressed in another chapter of this book.

## Activation of Caspases and Expression of Apoptotic and Anti-apoptotic Proteins in PDT Resistant Cells

Almeida et al. [85] analyzed the intracellular signaling mechanisms in PDT. They found that there are two major apoptotic pathways, both of which involved the activation of caspase 8 or 9 (initiators) leading to the activation of caspases 3, 6 and 7 (effector caspases). In addition, the lysosomal pathway is a prelude for the mitochondria-mediated apoptosis after lysosomal photodamage [81].

As mentioned before, monocationic PS such as monocationic porphyrin localize to the plasma membrane, and during the first minutes of irradiation, relocalized to the cytosol [75]. With longer periods of irradiation, procaspases -3 and -9 are affected by photodamage, preventing an apoptotic response. Although this is not a general rule for all the PS that initially bind to the plasma membrane, these results indicate that the absence of apoptosis can result from photodamage to keypoints of the apoptotic program.

There were no changes in caspase 8 RNA expression levels according to studies carried by Wild et al. [86] where they analyzed 3 cells lines: a normal cell line (UROtsa, urothelial) and two tumor cell lines (RT4, urothelial; HT29, colonic) following ALA-PDT. Although there were no changes at the RNA level, they found a delay in the activation of caspase 8 only in the normal cells, suggesting that this caspase pathway activation might be a secondary way to ensure photodamage. According to caspase -8, the active caspase 3 fragment was found only in the normal urothelial cell line 1 h after photodynamic therapy. Combined data analyses suggest that photodynamic therapy *in vitro* leads to apoptosis in UROtsa and to necrosis in the tumor cell lines, respectively. The activation of caspases 8, 3, 6 and 7 was found in HeLa cells treated with BPD-MA-PDT [87].

Ruhdorfer et al. [11] carried out gene expression analysis in squamous cell carcinoma A-431 after ALA-PDT and found that the product of the '*Fas-associated via death domain*' (FADD) gene was strongly induced. FADD is an adaptor molecule, which via its death effector domain (DED) engages the DEDs of procaspase 8, which will become caspase 8 and triggering the apoptotic pathway.

It has recently been published that the anti-apoptotic protein Mcl-1 plays an important role in protecting cells from PDT-induced apoptosis. In contrast to the reduction in the anti-apoptotic proteins Bcl-2 and Bcl-xl, sub-lethal PDT induces an increase in Mcl-1 expression. Silencing Mcl-1 sensitizes tumor cells to PDT-induced apoptosis, and ectopic expression of Mcl-1 significantly delays Bax translocation to the mitochondria and inhibits caspase3 activity following PDT [88]. Mcl-1 expression is associated closely with the activated AKT signaling following PDT. Treatment with Celecoxib, a non-steroidal anti-inflammatory drug, downregulated Mcl-1 expression, and enhanced PDT-induced apoptosis both *in vitro* and *in vivo*. This down-regulation is closely related to the inhibition effect of Celecoxib on the AKT/GSK-3β pathway, and was blocked upon addition of the GSK-3β inhibitor LiCl or the proteasome inhibitor MG132. In addition, a loss in Mcl-1 by inhibiting AKT promoted PDT-induced apoptosis through the mitochondrial pathway [88].

It was reported that it is possible to circumvent PDT resistance in an apoptosis-independent manner. Since it was found that PCI of gelonin was more effective in the PDT-resistant MES-SA/Dx5 cells as compared to the normal lines, death-inducing signaling was studied. A low activation of caspase3 and a strong PARP I cleavage after PCI occurred in both cell lines. The PARP I activation was, however, stronger after PCI than after PDT in the MES-SA cells, but not in the MES-SA/Dx5 cells and, therefore, cannot explain the strong PCI effect in the MES-SA/Dx5 cells [53].

The Bcl-2 family is a large family of proteins that play a key role in the apoptotic pathway. It is composed of anti-apoptotic members such as Bcl-2, CED-9 and Bcl-XL, and pro-apoptotic members such as Bak, Bax, BNIP3, as well as the BH3-only subfamily (Bik, Blk, Hrk, BimL, Bad, Bid) [89, 90].

Since Bcl-2 proteins are frequently activated after PDT-treatment [91, 92], these proteins may be involved in the development of cell resistance. Upon PDT, when photodamage targets the mitochondria and/or the endoplasmic reticulum, the anti-apoptotic protein Bcl-2 is affected, while lysosomal photodamage results in the activation of pro-apoptotic protein Bid, also leading to apoptosis [29].

Shen et al. [32] employed PDT-resistant HT29 cells to perform gene expression analysis of apoptosis- related genes. This study showed an increased expression of Bcl-2 and heat shock protein 27 as well as a decreased expression of Bax in the PDT resistant cells. However, mRNA and protein expression levels for the propaptotic BNIP3 were increased. The same group reported that these resistant cells showed a significant increase in cisplatin sensitivity that correlated with the increased BNIP3 and decreased mutant p53 protein levels [93].

HL60 cells transfected with Bcl-2 were able to suppress apoptosis after BPD-MA-PDT treatment [91]. They also found that overexpression of Bcl-2 or Bcl-XL did not avoid apoptosis by preventing the release of cytochrome C but instead by blocking the activation of caspases [87]. Also, CHO cells overexpressing Bcl-2 showed a 2-fold increase of PDT-resistance compared to the parental cells [94, 95]. In addition, human gastric adenocarcinoma MGC803 and A 431epidermoid carcinoma cells transfected with the Bcl-2 antisense reversed the resistance to PDT [92, 96].

There is, however, some controversy regarding the use of Bcl-2 expression as a predictor for PDT response. Different groups have used PDT-treated tumor biopsies to try to correlate Bcl-2 expression and the outcome of the treatment. Kawaguchi et al. [97] found no correlation between Bcl-2 or p53 expression and local recurrence after PDT in squamous cell carcinomas of the bronchus. McGarrity et al. [98] reported that variable changes in Bcl-2 and p53 immunoreactivity were noted in normal and carcinoma tissues biopsies of esophageal tumors treated with PII-PDT, whereas Koukourakis et al. suggested that Bcl-2 expression is associated with a favorable outcome of PDT, which could be due to the selective degradation of Bcl-2 induced by PDT, leading to apoptosis by decreasing the Bcl-2/Bax ratio [99]. In this context, several groups have shown in different cell lines, PDT-induced damage to Bcl-2.

Human breast cancer MCF-7c3 cells expressing procaspase-3, known to respond to PDT via apoptosis, were transfected with wild-type Bcl-2 or Bcl-2 deletion mutants lacking the anchorage region to the membrane, resulting in a relative resistance to Pc 4-PDT. These results suggested that the deleted regions, which include a caspase-3 cleavage site, are not necessary for the inhibition of PDT-induced apoptosis. On the other hand, a C-terminal truncated Bcl-2 mutant provided no protection, indicating that the degree of Bcl-2 photodamage determines the level of sensitivity of the cancer cells to apoptosis in response to PDT. In addition, overexpression of Bcl-2 in MCF-7c3 cells inhibited the activation-associated conformational change of the pro-apoptotic protein Bax, consequently leading to a requirement of higher light doses to activate Bax [100].

Employing the same concept, Chiu et al. [101] reported studies of the role of Bax in apoptosis and cell killing caused by PDT. MCF-7c3 cells treated with antisense Bax yielded a 50% inhibition of PDT-induced apoptosis. Similarly, in a human prostate cancer cell line DU-145, which does not express Bax, apoptosis was completely blocked after Pc 4-PDT treatment, but despite the lacking of Bax the cells were equally sensitive to PDT. Since cells deficient in Bax remain as photosensitive as Bax-proficient cells, the authors concluded that the commitment to cell death is likely determined before the step of Bax activation and cytochrome c release or is independent of them.

As mentioned before, Usuda et al. [102] showed that PII-PDT can induce damage to Bcl-2 and, therefore, apoptosis. Employing different PS targeting different organelles showed that targeting the mitochondria, as PII-PDT, is more effective in inducing apoptosis and Bcl-2 photodamage, compared to a lysosomal target agent such as NPe6-PDT. The latest PS showed no damage to Bcl-2 and a delayed apoptosis. Also, Bcl-2 overexpressing cells were considerably more resistant to NPe6-PDT than the parental MCF-7c3 cells.

Using ATX-s10, a PS which localizes to mitochondria and lysosomes, it was reported that overexpression of wild-type Bcl-2 conferred relative resistance to MCF-7 cells to PDT. Inhibition of lysosomal cathepsins B and D protected MCF-7c3 cells from apoptosis induced by ATX-s10-PDT. This demonstrated that lysosomal photodamage can initiate an apoptotic response and this apoptotic pathway can be regulated by photodamage to Bcl-2 via the mitochondria [103] Using the Tao variant of 1c1c.7 murine hepatoma cells having lysosomal fragility, apoptosis was delayed and diminished upon exposure to NPe6-PDT, thus exhibiting resistance to the treatment [104].

The tumor suppressor P53 is the most frequently mutated gene in human tumors. Increased P53 expression affects its target genes expression and leads to cell cycle arrest or apoptotic cell death depending on the cell type and context [105, 106]. P53-deficient cells are often less responsive to chemotherapeutics, and this implies that it could also be responsible for PDT resistance. However, no conclusive reports are available to show that the down-regulation of this protein may induce resistance to PDT. On the other hand, cells lacking functional p53 fail to undergo apoptosis,resulting in continued proliferation.

Using hypocrellin as PS it was found that wild-type p53 transfected-HT29 human colorectal carcinoma cells were 2-fold sensitive to PDT than the parentals [96]. In the same trend, human promyelocytic leukemia HL60 cells which express the wild type p53 were also more sensitive to PDT, with both PII and tin ethyl etiopurpurin I (SnET2), compared to cells presenting deletions or mutations in P53. The human colon carcinoma cell line LS513 which expresses wild-type p53 was also more sensitive to PII-PDT compared to the mutated P53 counterpart [107]. In every case, the cell lines underwent rapid apoptosis in response to PDT [108]. Immortalized fibroblasts derived from Li-Fraumeni syndrome patients, that only have one p53 allele and is mutated, were less sensitive to PII-PDT compared to normal fibroblasts [109]. On the other hand, expression of the oncoprotein E6 which suppresses p53 function did not alter the response to PDT in LS513 and MCF-7 cells [109]. However, in spite of all the evidence involving p53 on photodynamic sensitivity, there is yet no conclusive association of p53 with the PDT response [97, 99].

An interesting and unexpected finding was that PpIX interacts with wild-type p53 *in vitro* and induces cell death of colon cancer cells in a p53-dependent and independent manners. PpIX might directly target p53 and stabilize it in human colon cancer cells HCT116 probably by disrupting the p53/MDM2 complex. This might lead to p53-dependent cell death via p53-regulated apoptosis in the dark, prior to irradiation and also upon irradiation [110].

## Cellular Antioxidant Defense Mechanisms

It has been shown that the antioxidant defense mechanisms of the cell such as superoxide dismutases (SOD), the glutathione system and catalase or lipoamide dehydrogenase, antagonized the effects of PDT [111–114].

MCF-7 cells could be protected from PDT damage by transfecting them with the glutathione peroxidase gene, which helps with the removal of lipid hydroperoxides in living cells after $^1O_2$ exposure. In addition, it was found that human kidney 293 cells over-expressing glutathione S-transferase P1-1 had a reduced phototoxicity induced by Hypericin-PDT, by reversion of downregulation of the nutrient-sensing protein kinase mTOR and blocking apoptosis [115].

The glutathione system has been involved in chemoresistance [116]. In addition, a few authors reported that detoxification by glutathione conjugation was correlated with PDT resistance. Luna & Gomer [6] found an increase in the levels of reduced glutathione in their resistant cells RIF, but no alterations in the levels of glutathione peroxidase or superoxide. However, Singh et al. found no differences in glutathione levels in the RIF-8A resistant variant [35]. Our clones resistant to ALA-PDT [7] showed that the reduced glutathione content expressed on the basis of cell number increased two-fold, however, no differences were observed when expressed in per μg of protein, thus being difficult to evaluate the role of GSH detoxification in this system due to the different protein content in the resistant lines. However, the GSH:

porphyrins are higher in the resistant clones, suggesting an increased detoxifying activity per molecule of PS.

Mikesova et al. [117] compared the sensitivity of six colon-derived cancer cell lines to Hypericin-PDT, revealing a whole spectrum of responses from insignificant to high cytotoxicity. It was found that the cell line sensitivity was partially but not directly related to the intracellular Hypericin content, glutathione level or redox status, demonstrating partial but not direct correlation with resistance to PDT when considered separately but combination of these parameters are responsible for photocytotoxicity, thus reinforcing our theory of multifactorial features in the development of PDT resistance.

MES-SA/Dx5 cells resistant to DXR are cross-resistant to PDT. A DXR-induced increased expression of the ROS-scavenging proteins glutathione peroxidase GPx1 and GPx4 in MES-SA/Dx5 cells was indicated as the mechanism of resistance to PDT, in line with the reduction in PDT-generated ROS observed in this cell line. The MES-SA/Dx5 cells were also cross-resistant to ionizing radiation in agreement with the increased GPx1 and GPx4 expression [53].

In cancer cells resistant to DXR, overexpression of Glutathione transferase pi (GSTP1) is also suggested to influence the cellular redox status through the suppression of DXR conversion to semiquinone free radical and the subsequent production of ROS. Thus, the overexpression of GSTP1 is related to the development of drug resistance in cancer cells not only by increased detoxification of anticancer agents, but also by suppression of cellular ROS which induce cell death [118].

In the colon adenocarcinoma cell line LoVoDX resistant to DXR, oxidative changes induced by Photofrin-PDT were delayed in comparison to its DXR-sensitive counterpart LoVo. The expressions of GSTP1, a marker protein for photochemical toxicity, and secretory phospholipase A(2), a prognostic and diagnostic marker for colon cancers, were increased in both cell types after PDT. Increased SOD1 activity and TBARS levels in both cell lines, together with a decrease of protein-associated -SH groups were also evidenced. The increasing level of ROS following the oxidation of sulfhydryl cell groups and lipid peroxidation influence the activity of many transporters and enzymes [119].

Superoxide dismutases (SOD) are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide, and their action is relevant to photodamage [120, 121]. The expression of the isoform SOD2 was found to be differentially regulated by ALA-PDT. Up-regulation was found in the urothelial RT4 tumor cells, not affected in the colonic HT29 tumor cell line and slightly down-regulated in the normal urothelial cells UROtsa [86] after ALA-PDT treatment.

## Heat Shock Proteins in the Response to Photodamage

It is known that heat, chemical exposure, oxidation and PDT affect the expression of heat shock proteins (HSPs), which in turn are associated with modulation of cellular damage [9, 122]. HSP27, HSP34, HSP60, HSP70, HSP90, HSP110 [123, 124],

glucose regulated proteins GRPs (GRP74, GRP78, GRP94 and GRP100) [125] and heme oxygenase (HO-1) [126] have also been involved in protecting the cells from photodamage.

Luna & Gomer showed that their RIF PII-PDT-resistant cells increased the expression of HSP70 and H0-1 mRNA upon PDT treatment [6], without changing the expression of these proteins. The same group has previously reported that hyperthermia-resistant cells which overexpress HSP70 were not cross resistant to PDT [127].

Verwanger et al. [128] ran a cDNA array in human squamous cell carcinoma cells A-431 after treatment with ALA-PDT and found increased expression of HSP70. They also found increased expression of HO-1 following dark incubation with ALA, which was not further augmented after irradiation, which was ascribed to the need for heme degradation after PpIX cell loading.

HSP60, a chaperone found mainly in mitochondria, was shown to be overexpressed in colon cancer cells HT29 and in the PDT-resistant fibrosarcoma RIF-8A cells [129]. Using microarray analysis, the same group found that the mRNA of HSP27 was increased in these cells, which is known to be involved in the signaling pathway leading to apoptosis [130]. Moreover, cells stably transfected with HSP27 cDNA showed an increased survival after PII-PDT treatment, suggesting that this protein may play a key role in PDT resistance. Similarly, an increased expression of HSP27 mRNA was found in the HT29 human colon adenocarcinoma PDT-resistant cells [32].

HSP1 was also found to be phosphorylated and consequently activated after Pc 4-PDT of the mouse lymphoma L5178Y cells [131]. In the same way, several studies have shown that many stress response proteins are induced after PDT treatment [28]. The increased expression of HO-1 either by induction with hemin or by stable transfection of the gene, increased the resistance of colon adenocarcinoma C-26 tumor cells to PDT-mediated cytotoxicity. On the other hand, treatment of the cells with an HO-1 inhibitor augmented the rate of phototoxicity [132].

Treatment of cells with a calcium ionophore increased the expression of GRPs and also developed PDT resistance after long exposure to PII protocol (16 h) [125]. This study also indicated elevated levels of mRNA encoding GRP-78 and GRP-94 and an increase in GRP protein synthesis in RIF-1 cells even before irradiation, showing that the PS itself is capable of inducing an oxidative stress response. While a short exposure protocol (1 h) prior to illumination, resulted in minimal increase in GRP mRNA levels or GRP protein synthesis. These results suggest that subcellular localization of the PS, which is the factor mainly affected by time exposure, is correlated with GRP induction.

Since GRP78 mRNA and protein levels are elevated in response to PDT in various cancer cell lines, stable overexpression of GRP78 and its role in resistance to PDT was investigated. GRP78-targeting subtilase cytotoxin catalytic subunit fused with epidermal growth factor (EGF-SubA) sensitizes various cancer cells to Photofrin-mediated PDT. The combination treatment is cytotoxic to apoptosis-competent SW-900 lung cancer cells, as well as to Bax-deficient and apoptosis-resistant DU-145 prostate cancer cells. In these cells, PDT and EGF-SubA cytotoxin induce the

expression of an ER stress-associated apoptosis-promoting transcription factor. Although some apoptotic events such as disruption of the mitochondrial membrane and caspase activation are detected after PDT, there is no phosphatidylserine plasma membrane externalization or DNA fragmentation, suggesting that in DU-145 cells the late apoptotic events are missing. Moreover, in SW-900 cells, EGF-SubA cytotoxin potentiates PDT-mediated cell death but attenuates PDT-induced apoptosis. In addition, the cell death cannot be reversed by caspase inhibitor z-VAD, confirming that apoptosis is not a major cell death mode triggered by the combination therapy. Moreover, no typical features of necrotic or autophagic cell death are recognized. Instead, an extensive cellular vacuolation of ER origin is observed. Altogether, these findings indicate that PDT and GRP78-targeting cytotoxin treatment can efficiently kill cancer cells independent on their apoptotic competence and triggers an atypical, non-apoptotic cell death [133].

## Changes in Cytoskeleton, Cell to Cell Adhesion and Adhesion to Substrate in PDT Resistant Cells

The extracellular matrix (ECM) is the non-cellular component present within all tissues and organs, and provides not only essential physical scaffolding for the cellular constituents but also initiates crucial biochemical and biomechanical cues that are required for tissue morphogenesis, differentiation and homeostasis. Cell adhesion to the ECM is mediated by ECM receptors, such as integrins, discoidin domain receptors and syndecans. Adhesion mediates cytoskeletal coupling to the ECM and is involved in cell migration through the ECM. Migration, cell adhesion, cell-to-cell communication differentiation and survival are common functions of the ECM [134, 135].

Integrins are the major class of surface receptors that attach to the extracellular matrix (ECM) and are responsible for a cell's interaction with its environment; these receptors process external signals into intracellular ones and induce a number of regulatory cascades. Ultimately, this can lead to a variety of cellular responses. Integrins are non-covalently attached heterodimer transmembrane receptors that consist of α- and β-subunits, forming a functional receptor. Signals that come from intracellular receptors can regulate adhesion, migration, growth, differentiation, and death of cells [136, 137].

The chemotherapy resistance phenotype has been often associated with altered expression patterns of cytoskeletal components and adhesion [138, 139]. In recent years, it has been established that the cell-cell and cell-matrix interactions involve the reorganization of the cytoskeleton and also involved the activation of multiple signaling pathways that can modify cell growth, survival and differentiation. Experimental data provided evidence that the anti-apoptotic pathways mediated by cell adhesion induce tumor resistance to different injuries. Cell adhesion mediates drug resistance (CAM-DR) in multiple myeloma, malignant lymphoma, acute and chronic leukaemias, as well as in pancreatic cancer, neuroblastoma, small cell and

non-small cell lung cancer, mesothelioma, colorectal carcinoma, and breast cancer, and is based on the observation that cells that adhere to ECM ligands are protected from undergoing apoptosis. Cell adhesion protects from death by radiation, and genotoxic chemotherapy [140, 141]. Cell adhesion to ECM mediated by integrins impact favorably to both normal and tumor cells, and it has been implicated in the development of radioresistance. This phenomenon has been called cell adhesion-mediated radioresistance, and it can be reversed by overexpression of integrin-linked kinases, with concomitant reduction of cell size and adhesion to ECM proteins [142, 143].

Many changes related to cell adhesion have been reported as a consequence of photodamage, such as inhibition of cell adhesion by PDT-BPD-MA [144]. In addition, Verteporfin-PDT induced a transient decrease in adhesion of human ovarian cancer cells OVCAR 3 to collagen IV, laminin, fibronectin, and vitronectin. Interestingly, after photosensitization, the β1- integrin on the cell surface could still react with anti- β1 antibody suggesting that the subunit was still structurally intact, although β1-integrin-containing focal adhesion plaques (a functional attribute) became diffuse. The loss in integrin function arises largely through intracellular damage rather than through direct damage to the integrin proteins on the cell surface [145].

Tumor cells are in general less adhesive than normal cells, which contributes to tumor cell detachment and metastasis. In this sense, fibronectin and its integrin receptors play a key role in tumor development. Rudhorfer et al. [11] using a squamous carncinoma cell line A-431 observed that ALA-PDT markedly downregulated the fibronectin gene. As a result of this downregulation, cells rounding up and detachment begin to occur, and as a consequence, migration and metastasis are increased.

PDT employing PII and BPD-MA applied to colon carcinoma cells induced a transient decrease in adhesiveness and in adhesion molecules expression [146]. The authors suggested that the decrease in adhesiveness could account for the decreased metastatic potential of PDT-treated cancer cells. No matter what the effect is, there is an impact on the metastatic ability of the PDT-surviving cells. It could either increase [147] or decrease [148], and these differences may be due to the PS employed, the light dosage, cell model and even tumor location.

Although not yet well understood the effects of PDT on the ECM, it is clear that PDT induces changes in ECM. It has been described that PDT can either decrease or increase adhesion to plastic, ECM and to endothelial cells [145, 149, 150]. BPD-MA PDT inhibited cell adhesion of normal human fibroblasts, causing neither change in ECM nor integrin expression [144].

Overhaus et al. tested the hypothesis that PDT alters the vascular wall matrix thereby inhibiting invasive cell migration, and as such, provides an important barrier mechanism to favorably alter the vascular injury response. In an experimental intimal hyperplasia model composed of untreated smooth muscle cells and fibroblasts seeded on control and PDT-treated 3D collagen matrix gels, they demonstrated that PDT reduces the invasiveness degree of smooth muscle and fibroblast migration rate, generating a matrix barrier to invasive vascular cell migration [151].

The main components of the cytoskeleton are tubulin, actin and the intermediate filaments, and are one of the targets for PDT [152]. Changes in the cytoskeleton system have been related to tumor progression and metastasis [150], and it also favors changes in the cell shape during apoptosis by promoting the apoptotic bodies formation [153].

Signaling from the extracellular space such as changes in cell shape, or cell detachment, are sensed and transmitted into the intracellular space via p53 mitogen-activated protein kinase (MAPK), extracellular signal regulated kinases (ERK) 1/2 or JNK signaling pathways among others. It was found that promotion of p38MAPK, ERK, JNK and Ras signaling pathways supported survival and/or apoptosis after Hypericin-PDT treatment. This group also found that after PDT there was an up-regulation of NEDD9 (also called HEF1). This protein, part of the CAS protein family, localizes at focal adhesion sites. NEDD9 early up-regulation could participate in apoptosis induction and execution, activation of JNK kinases and in the transition of 'flat' attached cells to rounded mitotic cells [154].

Integrins not only regulate cell adhesion, but also participate in the crosstalk with different growth factor receptors. These receptors can be phosphorylated upon binding of their ligand or by the binding of integrins in the absence of ligand. Sanovic's group [154] also showed a downregulation of genes enconding for integrin 3, integrin β1, and integrin 6 after Hypericin-PDT, thus reducing the transduction of signals from the ECM and cell adhesion. They also found thrombospondin-1, which is a ligand for integrin β1 was downregulated in A-431 cells after treatment.

Buytaert et al. treated bladder cancer cells with Hypericin-PDT and they found a downregulation of integrin 2 and β3 precursors [155]. Studies conducted by Galaz et al. [156] reinforced the hypothesis that integrins downregulation induces cell detachment and apoptosis. They have demonstrated that altered levels of β1-integrins favored cell detachment and apoptosis via E-cadherin loss after ZnPc-PDT.

However, integrins are not the only proteins responsible for cell detachment and the control of rearrangements of the actin cytoskeleton, since Hypericin-PDT induce up-regulation of the Rho family GTPase 3 causing similar effects by inhibiting integrin-based focal adhesions and formation of actin stress fibers leading to cell rounding [154]. Sanovic's group has also reported another detachment mechanism affected by PDT which also contributes to significant changes in cell morphology and decreases cell adhesion, which is the overexpression of Pleckstrin homology-like domain, family A, member 1 (PHLDA1) [154].

Our group found that the ALA-PDT adenocarcinoma resistant cells *in vitro* were less invasive and tend to migrate less, while *in vivo* their ability to metastasize were decreased compared to the parental cell line. The lower tumor uptake, latency time and growth rate suggested that anchorage-dependent adhesion was also impaired *in vivo* in the resistant clones. However, *in vitro* binding to the ECM protein collagen I was higher for both of the clones, but no overexpression of β1 integrin was found, which is the main molecule involved in collagen I binding [157]. In addition to a loss of actin stress fibers, the resistant clones also exhibited disorganized actin cortical rim, as well as E-cadherin, β-catenin (cell–cell adhesion proteins) and vinculin (cytoskeleton-associated protein) distribution [157]. These alterations of

the cytoskeletal and adhesion proteins can be probably correlated with the lower metastatic phenotype of the cells *in vivo*.

Another major cytoskeletal protein is vimentin, which is degraded in response to various inducers of apoptosis [158, 159]. When Jurkat cells were transfected with a caspase-resistant vimentin variant, Phthalocyanine-PDT-induced apoptosis was partly suppressed and delayed, suggesting that this cytoskeleton component has a major role in the development of resistance by impairing caspase-3 translocation [160].

Cell attachment as well as cell to cell interactions may influence the plating efficiency of cells. Some of the PII-PDT resistant variants from Luna & Gomer showed a reduced plating efficiency of up to 36–43 % [6]. In addition, a much higher number of these resistant cells was required to generate a tumor when injected into syngeneic mice compared to non-resistant ones. In our hands, ALA-PDT variants resistant cells had shown an impaired plating efficiency that correlated with a lower tumor take when injected into mice. This characteristic may be associated to the ECM changes in the PDT resistant cells [149]. In addition, Perry et al. found a correlation between PII-PDT sensitivity and plating efficiency when analyzing an array of lung cell lines with different histologies [161].

Since the cellular shape is an important factor that regulates the cell sensitivity to mitogens, this suggests that the proliferative rate is anchorage dependent [162]. The ECM components on which the cells grow *in vivo* or the substract used to culture cells *in vitro*, commands the shape of the cells, as well as the production of specific proteins coming from external signals [163]. Since ALA-PDT resistant cells tend to spread more than the parental cell line, we hypotetized that these cells grown in suspension would loose the resistance [7]. However, in our study, the resistance indexes of cells growing in suspension did not change in the resistant clones as compared to the parental cells. Also, no significant effect was found when ALA-PDT was performed in cells growing onto fibronectin coatings.

Similarly to some studies that suggested the cell size could be related to resistance to chemotherapy [164], there are some evidences that cell size could also be related to PDT resistance. In the study carried out by Luna & Gomer the PII-PDT resistant variants obtained from RIF fibrosarcoma cells were larger and the protein content was increased [6]; similar results were obtained in the variants isolated by Sharkley et al. [30]. An increased cell spreading together with an increased number of cells per colony were also observed. Supporting this trend of thoughts, Richter et al. [165] treated several human leukemia cell lines with BPD-MA-PDT, in comparison to normal lymphocytes, they found that the resistance was related to the cell sizes, with the smallest cells being the most vulnerable. Our group also found that ALA-PDT resistant cells had twice the volume and protein content increase compared to the parental line [7]. Since the plasma membrane is the main target for PDT damage [206], a larger cell exposes a greater surface area, suggesting the effectiveness of the treatment could be inferior in the resistant clones.

## Nitric Oxide

As a general rule, depending on the cell type and the pathology, the gaseous radical nitric oxide (NO), could play either a protective or a toxic role in the cells. However, several studies have shown NO can induce many pathways to mediate chemoresistance. Inhibition of the proto-oncogene MYCN (v-myc myelocytomatosis viral related *oncogene*, neuroblastoma derived (avian)) and expression of a large set of ATP binding cassette transporters by NO, influence the chemoresistance outcome in neuroblastoma cells [166]. In malignant astrocytes, NO has been found to modulate radioresistance and chemoresistance against nitrosourea derivatives [167]. Using a blocking agent against all NO synthases reversed the resistant phenotype through induction of apoptosis in cisplatin-resistant ovarian cancer cells [168].

NO is not an effective oxidant *per se*, but under biological conditions could be converted to strong damaging oxidants. At low concentrations NO may act as an antioxidant in lipid membranes by scavenging chain propagating oxyl and peroxyl radicals [169], contributing to the overall cellular resistance to peroxidative stress. When this occurs during PDT, the outcome of the treatment may be impaired, even under nontoxic levels of exogenous NO as it was shown by Niziolek et al. [170] on PpIX-sensitized liposomes and breast tumor COH-BR1 cells treated with ALA-PDT.

iNOS (inducible nitric oxide synthase) modulates survival to confer chemoresistance in head and neck cancer [171]. The cytoprotective effects of NO in response to oxidative stress could be long-term in an indirect way. Cytokines can induce nitric oxide synthase to produce endogenous NO, conferring long-term hype in resistance to $H_2O_2$ or high-level NO cytotoxicity in hepatocytes [172, 173].

Resistance to ALA-PDT could be evidenced after rather long periods after exposure to NO; between 8 and 20 h after treatment of COH-BR1 tumor cells with the NO donor spermine NONOate (SPNO). A concomitant increase in HO-1 and ferritin levels was also observed. These cells exhibited an immediate radical-quenching effect of NO, but it also evoked a delayed cytoprotective response, suggesting that the protective mechanism involves the mobilization of "signaling" iron [174]. The same group reported in 2010 [175] that NO has the capacity to support apoptosis, and that the NO donor SPNO was able to inhibit necrosis but support apoptosis when cells were exposed to it before PDT. These observations were along with an increased activation of caspases-3 and -7. The effect of SPNO-supported apoptosis was more evident when comparing cells growing in glucose-deprived conditions to glucose-containing medium. They suggested that PDT resistant cells based their resistance on the membrane protection by NO and the maintenance of sufficient glycolytic ATP to sustain apoptosis.

The same group demonstrated that incubating lymphocytic leukemia L1210 cells with SPNO either immediately before or after light exposure PDT employing merocyanin 540 (a lipophilic dye that localizes primarily in the plasma membrane), photodamage was inhibited. They postulated that chain peroxidation triggered by iron-catalyzed turnover of nascent hydroperoxides generated by singlet oxygen attack on membrane lipids contributes significantly to phototoxicity, and that NO,

thus, acts cytoprotectively. Propagating radicals such as 5alpha-OOH are impaired by action of NO on photodamaged cells [176].

In our laboratory [177], we generated NO-resistant cells of murine the LM3 mouse breast adenocarcinoma called LM3-SNP, by successive exposures to the NO donor sodium nitroprusside (SNP). We have found this variant had no cross-resistance to ALA-PDT treatment. In parallel, we have also induced ALA-PDT resistance in these NO-resistant LM3-SNP cells, suggesting that resistance to NO did not interfere in the development of PDT resistance. Moreover, we found that several cell lines with different NO production levels responded in a similar way to ALA-PDT treatment [178], and that the modulation of NO levels did not modify the intrinsic response of various cells lines to PDT treatment.

Bhowmick et al. [179] reported that iNOS up-regulation after PDT treatment, induced an increase of tumor cell resistance. The inducible form iNOS was found to be up-regulated in breast tumor COH-BR1 cells after ALA-PDT treatment, while the other NOS isoforms nNOS and eNOS were unaffected. Also incubating the cells with L-NAME (NG-Nitro-L-arginine methyl ester), an NOS inhibitor, during PDT enhanced the activation of caspases-3 and -7 and apoptotic killing, suggesting that iNOS was acting as photoprotective. In line with these findings, exposure to the NO scavenger cPTIO enhanced ALA-PDT-induced caspases-3 and -7 activation and apoptotic death in the mentioned breast tumor cells.

Similar results were found by the same authors in prostate cancer PC-3 cells treated with PDT, which resulted in upregulation of iNOS, as well as caspases-3 and -7 activation and apoptosis stimulation after treatment with iNOS inhibitors and an NO scavenger. Cells surviving PDT had an increased cell cycle, but iNOS inhibition prevented this and the increase in cell cycle S-phase occupancy observed after irradiation, showing that upregulation of NOS/NO elicited both a pro-survival and pro-growth response. This was the first report of NO-dependent growth stimulation in cancer cells exposed to a PDT oxidative stress. The pro-survival response described could be a general phenomenon in NOS-expressing tumors subjected to PDT, and one that might seriously compromise treatment effectiveness unless counteracted. It has been proposed the use of the iNOS inhibitor GW274150 to overcome iNOS-mediated resistance to PDT [180].

## Hypoxia

It is well known that most tumors undergo hypoxia, which is a therapeutic challenge since it can reduce the effectiveness of radiotherapy and PDT [181]. Tumor hypoxia can also induce proteomics and genomic changes in the cancer cells, therefore negatively affecting the therapeutic outcome. Tumor hypoxia influences the selection of malignant cells which have been able to overcome the microenvironment of nutrient deprivation, this making the tumor more aggressive and promoting the development of a more treatment-resistant disease.

PDT induces vascular damage and oxygen consumption resulting in tissue hypoxia which in turn may limit the efficacy of this treatment. Tumor cells may protect themselves against PDT-mediated damage by stabilizing the hypoxia-inducible factor 1 (HIF1)-alpha [99, 182]. It has been reported that PDT induces hypoxia and the expression of vascular endothelial growth factor (VEGF) via the HIF1-alpha pathway, which in turn promotes angiogenesis, thus enhancing tumor proliferation and survival. VEGF mRNA expression was induced in the lung cancer cell line SBC-3 after ATX-s10-PDT [183]. When the human esophageal cell line Het-1A was induced to overexpress HIF-1alpha, it displayed resistance to ALA-PDT. Moreover, knocking down of the HIF-1alpha restored the photosensitivity of the cells [184].

In order to overcome PDT resistance induced by hypoxia, some strategies have been developed. Through a concept called "arterial flow focalization", by controlled temporary endo or peri-vascular occlusion of the collateral arterial branch(es) upstream of the tumor, it is possible to redirect blood flow through the principal artery of the downstream tumor (organ), thereby increasing tumor arterial flow, and hence oxygen supply, thus increasing tumor PtO2 at the desired intensity and timing, in synchrony with radiotherapy, and greatly improving radiosensitivity. Chemotherapy and photodynamic therapy efficacy could be also be increased, by increasing the PtO2 and by improvement of tumor blood perfusion and hence drug delivery to the tumor [185]. Additionally, the more hypoxic cells can be preferentially targeted by bio-reductive drugs and hypoxia-directed gene therapy [186, 187].

Using a synthetic self-assembling peptide as a cellular scaffold, Alemany-Ribes et al. [188] recreated the *in vivo* limitation of oxygen and drug diffusion and its biological effect, which is the development of cellular resistance to therapy. Cells grown in the 3D cultures upregulated the expression of the hypoxia-responsive genes EGF and insulin-like growth factor binding protein 3 (IGFBP3), which act as oxygen markers. 3D cultures were found to be more resistant to PDT than traditional 2D monolayers, exhibiting a radial survival pattern in which the core of the construct maintained a larger percentage of living cells. After circulating oxygen stream during irradiation, complete cell death was observed under conditions in which cell viability had been 80% in the absence of oxygen flow. These data indicate that the high cellular survival observed under static conditions was due to the low oxygen concentration in the core of the construct, which created a protective microenvironment for cells, thus reinforcing the importance of cell hypoxia in the outcome of PDT

## Conclusions and Future Directions

Recently, in addition to the development of PDT resistant cells as models to further study the impact of PDT on cellular targets, there have been an increasing interest in developing techniques related to the use of PS and light to overcome drug resistance to chemotherapeutic drugs. One of the mechanisms of the reversion of MDR takes advantage of the acidic microenvironment within drug vesicles.

Photochemical-mediated release of chemotherapeutic agents that are either targets or not targets of P-gp pumps, which are trapped in endocytic vesicles, has been shown to reverse the MDR phenotype. This approach can be employed for the treatment of any chemoresistant cell, but particularly cancer stem cells. In addition, illumination of ABCG2-rich extracellular vesicles that accumulate photosensitive cytotoxic drugs has been demonstrated to overcome resistance. Another approach uses nanomedicinal tools to deliver the PS more efficiently and to reduce the chances of being pumped out from the cytoplasm, e.g., pH-sensitive nanoparticles that enable pH-dependent PDT.

Combinations of PDT and targeted therapies, as well as the use of PS delivered in nanoparticles such as poly ethylene glycol-based, carbon nanotubes, dendrimers, carbon-based nanoparticles and polymeric micelles, are the most recent strategies to circumvent photoresistance and will be discussed in the next chapters.

**No conflict statement** **"**No potential conflicts of interest were disclosed."

## References

1. Gomer CJ. Photodynamic therapy. Oxford: Pergamon Press; 1987.
2. Dougherty TJ. Photodynamic therapy-new approaches. Semin Surg Oncol. 1989;5:6–16.
3. Weishaupt K, Gomer C, Dougherty T. Identification of singlet oxygen as the cytotoxic agent in photoactivation of a murine tumor. Cancer Res. 1976;36:2326–9.
4. Mayhew S, Vernon D, Schofield J, Griffiths J, Brown S. Investigation of cross-resistance to a range of photosensitizers, hyperthermia and UV light in two radiation-induced fibrosarcoma cell strains resistant to photodynamic therapy in vitro. Photochem Photobiol. 2001;73:39–46.
5. Singh G, Wilson B, Sharkey S, Browman G, Deschamps P. Resistance to photodynamic therapy in radiation induced fibrosarcoma-1 and chinese hamster ovary-multi-drug resistant cells in vitro. Photochem Photobiol. 1991;54:307–12.
6. Luna M, Gomer C. Isolation and initial characterization of mouse tumor cells resistant to porphyrin-mediated photodynamic therapy. Cancer Res. 1991;51:4243–9.
7. Casas A, Perotti C, Ortel B, Di Venosa G, Saccoliti M, Batlle A, Hasan T. Tumor cell lines resistant to ALA-mediated photodynamic therapy and possible tools to target surviving cells. Int J Oncol. 2006;29:397–405.
8. Henderson B, Miller A. Effects of scavengers of reactive oxygen and radical species on cell survival following photodynamic treatment in vitro: comparison to ionizing radiation. Radiat Res. 1986;108:196–205.
9. Gomer C, Ryter S, Ferrario A, Rucker N, Wong S, Fisher A. Photodynamic therapy-mediated oxidative stress can induce the expression of heat shock proteins. Cancer Res. 1996;56:2355–60.
10. Shackley DC, Haylett A, Whitehurst C, Betts CD, O'Flynn K, Clarke NW, Moore JV. Comparison of the cellular molecular stress responses after treatments used in bladder cancer. BJU Int. 2002;90:924–32.
11. Ruhdorfer S, Sanovic R, Sander V, Krammer B, Verwanger T. Gene expression profiling of the human carcinoma cell line A-431 after 5-aminolevulinic acid-based photodynamic treatment. Int J Oncol. 2007;30:1253–62.
12. Morgan J, Potter WR, Oseroff AR. Comparison of photodynamic targets in a carcinoma cell line and its mitochondrial DNA-deficient derivative. Photochem Photobiol. 2000;71:747–57.
13. Peng Q, Moan J, Farrants GW, Danielsen HE, Rimington C. Location of P-II and AlPCS4 in human tumor LOX in vitro and in vivo by means of computer-enhanced video fluorescence microscopy. Cancer Lett. 1991;58:37–47.

14. Lin CW, Shulok JR, Kirley SD, Cincotta L, Foley JW. Lysosomal localization and mechanism of uptake of Nile blue photosensitizers in tumor cells. Cancer Res. 1991;51:2710–9.
15. Van Hillesberg R, van der Berg J, Kort W, Terpstra O, Wilson J. Selective accumulation of endogenously produced porphyrins in a liver metastasis model in rats. Gastroenterology. 1992;103:647–51.
16. Madsen SJ, Sun CH, Tromberg BJ, Hirschberg H. Repetitive 5-aminolevulinic acid-mediated photodynamic therapy on human glioma spheroids. J Neurooncol. 2003; 62:243–50.
17. Casas A, Di Venosa G, Hasan T, Al Batlle. Mechanisms of resistance to photodynamic therapy. Curr Med Chem. 2011;18:2486–515.
18. Luna M, Ferrario A, Rucker N, Gomer C. Decreased expression and function of alpha-2 macroglobulin receptor/low density lipoprotein receptor-related protein in photodynamic therapy-resistant mouse tumor cells. Cancer Res. 1995;55:1820–3.
19. Wilson B, Olivo M, Singh G. Subcellular localization of photofrin and aminolevulinic acid and photodynamic cross-resistance in vitro in radiation-induced fibrosarcoma cells sensitive or resistant to photofrin-mediated photodynamic therapy. Photochem Photobiol. 1997;65:166–76.
20. Hilf R, Smail D, Murant R, Leakey P, Gibson S. Hematoporphyrin derivative induced photosensitivity of mitochondrial succinate dehydrogenase and selected cytosolic enzymes of R3230AC mammary adenocarcinomas of rats. Cancer Res. 1984;44:1483–88.
21. Nelson JS, Sun CH, Berns MW. Study of the in vivo and in vitro photosensitizing capabilities of uroporphyrin I compared to photofrin II. Lasers Surg Med. 1986;6:131–6.
22. Okunaka T, Eckhauser ML, Kato H, Bomaminio A, Yamamoto H. Aizawa K, Sarasua MM, Koehler KA. Correlation between photodynamic efficacy of differing porphyrins and membrane partitioning behavior. Lasers Surg Med. 1992;12:98–103.
23. Strauss WS, Sailer R, Schneckenburger H, Akgun N, Gottfried V, Chetwer L, Kimel S. Photodynamic efficacy of naturally occurring porphyrins in endothelial cells in vitro and microvasculature in vivo. J Photochem Photobiol. 1997;39:176–84.
24. Musser DA, Oseroff AR. The use of tetrazolium salts to determine sites of damage to the mitochondrial electron transport chain in intact cells following in vitro photodynamic therapy with Photofrin II. Photochem Photobiol. 1994;59:621–6.
25. Hilf R. Mitochondria are targets of photodynamic therapy. J Bioenerg Biomembr. 2007;39:85–9.
26. Boegheim JP, Lagerberg JW, Dubbelman TM, Tijssen K, Tanke HJ, Van Der Meulen J, Van Steveninck J. Photodynamic effects of hematoporphyrin derivative on the uptake of rhodamine 123 by mitochondria of intact murine L929 fibroblasts and Chinese ovary K1 cells. Photochem Photobiol. 1988;48:613–20.
27. Salet C, Moreno G. Photosensitization of mitochondria. Molecular and cellular aspects. J Photochem Photobiol B. 1990;5:133–50.
28. Moor AC. Signaling pathways in cell death and survival after photodynamic therapy. J Photochem Photobiol B. 2000;57:1–13.
29. Kessel D. Death pathways associated with photodynamic therapy. Med Laser Appl. 2006;21:219–24.
30. Sharkey S, Wilson B, Moorehead R, Singh G. Mitochondrial alterations in photodynamic therapy-resistant cells. Cancer Res. 1993;53:4994–9.
31. Crawford DR, Wang Y, Schools GP, Kochheiser J, Davies KJ. Down-regulation of mammalian mitochondrial RNAs during oxidative stress. Free Radic Biol Med. 1997;22:551–9.
32. Shen XY, Zacal N, Singh G, Rainbow AJ. Alterations in mitochondrial and apoptosis-regulating gene expression in photodynamic therapy-resistant variants of HT29 colon carcinoma cells. Photochem Photobiol. 2005;81:306–13.
33. Singh K, Russell J, Sigala B, Zhang Y, Williams J, Keshav K. Mitochondrial DNA determines the cellular response to cancer therapeutic agents. Oncogene. 1999;18:6641–6.
34. Wang X. The expanding role of mitochondria in apoptosis. Genes Dev. 2001;15:2922–33.
35. Moorehead R, Armstrong S, Wilson B, Singh G. Cross-resistance to cisplatin in cells resistant to photofrin-mediated photodynamic therapy. Cancer Res. 1994;54:2556–9.

36. Kessel D, Woodburn K, Skalkos D. Impaired accumulation of a cationic photosensitizing agent by a cell line exhibiting multidrug resistance. Photochem Photobiol. 1994;60:61–3.
37. Kessel D, Erickson C. Porphyrin photosensitization of multi-drug resistant cell types. Photochem Photobiol. 1992;55:397–9.
38. Tsai T, Hong RL, Tsai JC, Lou PJ, Ling IF, Chen, CT. Effect of 5-aminolevulinic acid-mediated photodynamic therapy on MCF-7 and MCF-7/ADR cells. Lasers Surg Med. 2004;34:62–72.
39. Robey RW, Steadman K, Polgar O, Bates SE. ABCG2-mediated transport of photosensitizers: potential impact on photodynamic therapy. Cancer Biol Ther. 2005;4:187–94.
40. Li W, Zhang W, Ohnishi K, Yamada I, Ohno R, Hashimoto K. 5-aminolevulinic acid-mediated photodynamic therapy in multidrug resistant leukemia cells. J Photochem Photobiol. 2001;60:79–86.
41. Chu ES, Yow CM, Shi M, Ho RJ. Effects of photoactivated 5-aminolevulinic acid hexyl ester on MDR1 over-expressing human uterine sarcoma cells. Toxicol Lett. 2008;181:7–12.
42. Tang PM, Zhang DM, Xuan NH, Tsui SK, Waye MM, Kong SK, Fong WP, Fung KP. Photodynamic therapy inhibits P-glycoprotein mediated multidrug resistance via JNK activation in human hepatocellular carcinoma using the photosensitizer pheophorbide a. Mol Cancer. 2009;8:56–68.
43. Selbo PK, Weyergang A, Bonsted A, Bown SG, Berg K. Photochemical internalization of therapeutic macromolecular agents: a novel strategy to kill multidrug-resistant cancer cells. J Pharmacol Exp Ther. 2006;319:604–12.
44. Merlin JL, Gautier H, Barberi-Heyob M, Teiten MH, Guillemin F. The multidrug resistance modulator SDZ-PSC 833 potentiates the photodynamic activity of chlorin e6 independently of P-glycoprotein in multidrug resistant human breast adenocarcinoma cells. Int J Oncol. 2003;22:733–9.
45. Teiten M, Bezdetnaya L, Merlin J, Bour-Dill C, Pauly M, Dicato M, Guillemin F. Effect of meta-tetra(hydrophenyl)chlorin (mTHPC)-mediated photodynamic therapy on sensitive and multidrug-resistant human breast cancer cells. J Photochem Photobiol B. 2001;62:146–52.
46. Cheung KK, Chan JY, Fung KP. Antiproliferative effect of pheophorbide a-mediated photodynamic therapy and its synergistic effect with doxorubicin on multiple drug-resistant uterine sarcoma cell MES-SA/Dx5. Drug Chem Toxicol. 2013;36:474–83.
47. Chu ES, Yow CM. Modulation of telomerase and signal transduction proteins by hexyl-ALA-photodynamic therapy (PDT) in human doxorubicin resistant cancer cell models. Photodiagnosis Photodyn Ther. 2012;9:243–55.
48. Diez B, Ernst G, Teijo MJ, Batlle A, Hajos S, Fukuda H. Combined chemotherapy and ALA-based photodynamic therapy in leukemic murine cells. Leuk Res. 2012;36:1179–84.
49. Preise D, Mazor O, Koudinova N, Liscovitch M, Scherz A, Salomon Y. Bypass of tumor drug resistance by antivascular therapy. Neoplasia. 2003;5:475–80.
50. Lou PJ, Lai PS, Shieh MJ, MacRobert AJ, Berg K, Bown SG. Reversal of doxorubicin resistance in breast cancer cells by photochemical internalization. Int J Cancer. 2006;119:2692–8.
51. Adigbli DK, Wilson DG, Farooqui N, Sousi E, Risley P, Taylor I, Macrobert AJ, Loizidou M. Photochemical internalisation of chemotherapy potentiates killing of multidrug-resistant breast and bladder cancer cells. Br J Cancer. 2007;97:502–12.
52. Berg K, Selbo PK, Prasmickaite L, Tjelle TE, Sandvig K, Moan J, Gaudernack G, Fodstad O, Kjølsrud S, Anholt H, Rodal GH, Rodal SK, Høgset A. Photochemical internalization: a novel technology for delivery of macromolecules into cytosol. Cancer Res. 1999;59:1180–3.
53. Olsen CE, Berg K, Selbo PK, Weyergang A. Circumvention of resistance to photodynamic therapy in doxorubicin-resistant sarcoma by photochemical internalization of gelonin. Free Radic Biol Med. 2013;65:1300–9.
54. Ling D, Park W, Park SJ, Lu Y, Kim KS, Hackett MJ, Kim BH, Yim H, Jeon YS, Na K, Hyeon T. Multifunctional tumor pH-Sensitive self-assembled nanoparticles for bimodal imaging and treatment of resistant heterogeneous tumors. J Am Chem Soc. 2014 [Epub ahead of print]
55. Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, Ross DD. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci USA. 1998;95:15665–70.

56. Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V, Dean M. A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. Cancer Res. 1998;58:5337–9.
57. Haimeur A, Conseil G, Deeley RG, Cole SP. The MRP-related and BCRP/ABCG2 multidrug resistance proteins: biology, substrate specificity and regulation. Curr Drug Metab. 2004;5:21–53.
58. Doyle LA, Ross DD. Multidrug resistance mediated by the breast cancer resistance protein BCRP (ABCG2). Oncogene. 2003;22:7340–58.
59. Selbo PK, Weyergang A, Eng MS, Bostad M, Mælandsmo GM, Høgset A, Berg K. Strongly amphiphilic photosensitizers are not substrates of the cancer stem cell marker ABCG2 and provides specific and efficient light-triggered drug delivery of an EGFR-targeted cytotoxic drug. J Control Release. 2012;159:197–203.
60. Navone N, Polo C, Frisardi A, Batlle A. Mouse mammary carcinoma PBGase and hydroxymethylbilane synthetase. Comp Biochem Physiol B. 1991;98:67–71.
61. Calzavara-Pinton P. Repetitive photodynamic therapy with topical delta-aminolaevulinic acid as an appropriate approach to the routine treatment of superficial non-melanoma skin tumors. J Photochem Photobiol B. 1995;29:53–7.
62. Jonker JW, Buitelaar M, Wagenaar E, Van Der Valk MA, Scheffer GL, Scheper RJ, Plosch T, Kuipers F, Elferink RP, Rosing H, Beijnen JH, Schinkel AH. The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. Proc Natl Acad Sci USA. 2002;99:15649–54.
63. Tsunoda Y, Usuda J, Imai K, Kubota M, Maehara S, Ohtani K. The expression of BCRP/ABCG2 causes resistance to Photofrin-PDT. Jpn J Laser Surg Med. 2008;28:355–61.
64. Robey RW, Steadman K, Polgar O, Morisaki K, Blayney M, Mistry P, Bates SE. Pheophorbide a is a specific probe for ABCG2 function and inhibition. Cancer Res. 2004;64:1242–6.
65. Moitra K, Lou H, Dean M. Multidrug efflux pumps and cancer stem cells: insights into multidrug resistance and therapeutic development. Clin Pharmacol Ther. 2011;89:491–502.
66. Zhou S, Schuetz JD, Bunting KD, Colapietro AM, Sampath J, Morris JJ, Lagutina I, Grosveld GC, Osawa M, Nakauchi H, Sorrentino BP. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med 2001;7:1028–34.
67. Gupta N, Martin PM, Miyauchi S, Ananth S, Herdman AV, Martindale RG, Podolsky R, Ganapathy V. Down-regulation of BCRP/ABCG2 in colorectal and cervical cancer. Biochem Biophys Res Commun. 2006;343:571–7.
68. Liu W, Baer MR, Bowman MJ, Pera P, Zheng X, Morgan J, Pandey RA, Oseroff AR. The tyrosine kinase inhibitor imatinib mesylate enhances the efficacy of photodynamic therapy by inhibiting ABCG2. Clin Cancer Res. 2007;13:2463–70.
69. Usuda J, Tsunoda Y, Ichinose S, Ishizumi T, Ohtani K, Maehara S, Ono S, Tsutsui H, Ohira T, Okunaka T, Furukawa K, Sugimoto Y, Kato H, Ikeda N. Breast cancer resistant protein (BCRP) is a molecular determinant of the outcome of photodynamic therapy (PDT) for centrally located early lung cancer. Lung Cancer. 2009;67:198–204.
70. Jendzelovský R, Mikes J, Koval' J, Soucek K, Procházková J, Kello M, Sacková V, Hofmanová J, Kozubík A, Fedorocko P. Drug efflux transporters, MRP1 and BCRP, affect the outcome of hypericin-mediated photodynamic therapy in HT-29 adenocarcinoma cells. Photochem Photobiol Sci. 2009;8:1716–23.
71. Krishnamurthy P, Ross DD, Nakanishi T, Bailey-Dell K, Zhou S, Mercer KE, Sarkadi B, Sorrentino BP, Schuetz JD. The stem cell marker Bcrp/ABCG2 enhances hypoxic cell survival through interactions with heme. J Biol Chem. 2004;279:24218–25.
72. Goler-Baron V, Assaraf YG. Structure and function of ABCG2-rich extracellular vesicles mediating multidrug resistance. PLoS One. 2011;6(1):e16007.
73. Adar Y, Stark M, Bram EE, Nowak-Sliwinska P, van den Bergh H, Szewczyk G, Sarna T, Skladanowski A, Griffioen AW, Assaraf YG. Imidazoacridinone-dependent lysosomal photodestruction: a pharmacological Trojan horse approach to eradicate multidrug-resistant cancers. Cell Death Dis. 2012;3:e293.

74. Oleinick NL, Morris RL, Belichenko I. The role of apoptosis in response to photodynamic therapy: what, where, why, and how. Photochem Photobiol Sci. 2002;1:1–21.
75. Kessel D. Relocalization of cationic porphyrins during photodynamic therapy. Photochem Photobiol Sci. 2002;1:837–40.
76. Reiners JJ Jr., Agostinis P, Berg K, Oleinick NL, Kessel D. Assessing autophagy in the context of photodynamic therapy. Autophagy. 2010;6:7–18.
77. Kessel D, Luo Y, Deng Y, Chang CK. The role of subcellular localization in initiation of apoptosis by photodynamic therapy. Photochem Photobiol. 1997;65:422–6.
78. Dellinger M. Apoptosis or necrosis following Photofrin photosensitization: influence of the incubation protocol. Photochem Phorobiol. 1996;64:182–7.
79. Kessel D, Castelli M. Evidence that Bcl-2 is the target of three photosensitizers that induce a rapid apoptotic response. Photochem Photobiol. 2001;74:318–22.
80. Kessel D, Luguya R, Vicente MG. Localization and photodynamic efficacy of two cationic porphyrins varying in charge distribution. Photochem Photobiol. 2003;78:431–5.
81. Kessel D, Luo Y, Mathieu P, Reiners JJ Jr. Determinants of the apoptotic response to lysosomal photodamage. Photochem Photobiol. 2000;71:196–200.
82. Kessel D, Oleinick NL. Initiation of autophagy by photodynamic therapy. Methods Enzymol. 2009;453:1–16.
83. Kessel D, Arroyo AS. Apoptotic and autophagic responses to Bcl-2 inhibition and photodamage. Photochem Photobiol Sci. 2007;6:1290–5.
84. Kessel D, Oleinick NL. Photodynamic therapy and cell death pathways. Methods Mol Biol. 2010;635:35–46.
85. Almeida RD, Manadas BJ, Carvalho AP, Duarte CB. Intracellular signaling mechanisms in photodynamic therapy. Biochim Biophys Acta. 2004;1704:59–86.
86. Wild PJ, Krieg RC, Seidl J, Stoehr R, Reher K, Hofmann C, Louhelainen J, Rosenthal A, Hartmann A, Pilarsky C, Bosserhoff AK, Knuechel R. RNA expression profiling of normal and tumor cells following photodynamic therapy with 5-aminolevulinic acid-induced protoporphyrin IX in vitro. Mol Cancer Ther. 2005;4:516–28.
87. Granville DJ, Carthy CM, Jiang H, Shore GC, McManus BM, Hunt DW. Rapid cytochrome c release, activation of caspases 3, 6, 7 and 8 followed by Bap31 cleavage in HeLa cells treated with photodynamic therapy. FEBS Lett. 1998;437: 5–10.
88. Song J, Chen Q, Xing D. Enhanced apoptotic effects by downregulating Mcl-1:evidence for the improvement of photodynamic therapy with Celecoxib. Exp Cell Res. 2013;319:1491–504.
89. Reed JC. Bcl-2 family proteins. Oncogene. 1998;17:3225–36.
90. Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. Genes. 1999;13:1899–911.
91. Granville DJ, Jiang H, An MT, Levy JG, McManus BM, Hunt DW. Bcl-2 overexpression blocks caspase activation and downstream apoptotic events instigated by photodynamic therapy. Br J Cancer. 1999;79:95–100.
92. Srivastava M, Ahmad H, Gupta S, Mukhtar H. Involvement of Bcl-2 and Bax in photodynamic therapy mediated apoptosis. J Biol Chem. 2001;276:15481–8.
93. Zacal N, Espiritu M, Singh G, Rainbow AJ. Increased BNip3 and decreased mutant p53 in cisplatin-sensitive PDT-resistant HT29 cells. Biochem Biophys Res Commun. 2005;331:648–57.
94. He J, Agarwal M, Larkin H, Friedman L, Xue L, Oleinick N. The induction of partial resistance to photodynamic therapy by the protooncogene BCL-2. Photochem Photobiol. 1996;64:845–52.
95. Kim H, Luo Y, Li G, Kessel D. Enhanced apoptotic response to photodynamic therapy after bcl-2 transfection. Cancer Res. 1999;59:3429–32.
96. Zhang WG, Ma LP, Wang SW, Zhang ZY, Cao GD. Antisense bcl-2 retrovirus vector increases the sensitivity of a human gastric adenocarcinoma cell line to photodynamic therapy, Photochem Photobiol. 1999;69:582–6.

97. Kawaguchi T, Yamamoto S, Naka N, Okishio K, Atagi S, Ogawara M, Hosoe S, Kawahara M, Furuse K. Immunohistochemical analysis of Bcl-2 protein in early squamous cell carcinoma of the bronchus treated with photodynamic therapy. Br J Cancer. 2000;82:418–23.
98. McGarrity TJ, Peiffer LP, Granville DJ, Carthy CM, Levy JG, Khandelwal M, Hunt DW. Apoptosis associated with esophageal adenocarcinoma: influence of photodynamic therapy. Cancer Lett. 2001;163:33–41.
99. Koukourakis M, Corti L, Skarlatos J, Giatromanolaki A, Krammer B, Blandamura S, Piazza M, Verwanger T, Schnitzhofer G, Kostandelos J, Beroukas K. Clinical and experimental evidence of Bcl-2 involvement in the response to photodynamic therapy. Antinacer Res. 2001;21:663–8.
100. Usuda J, Azizuddin K, Chiu SM, Oleinick NL. Association between the photodynamic loss of Bcl-2 and the sensitivity to apoptosis caused by phthalocyanine photodynamic therapy. Photochem Photobiol. 2003;78:1–8.
101. Chiu SM, Xue LY, Usuda J, Azizuddin K, Oleinick NL. Bax is essential for mitochondrion-mediated apoptosis but not for cell death caused by photodynamic therapy. Br J Cancer. 2003;89:1590–7.
102. Usuda J, Hirata T, Ichinose S, Ishizumi T, Inoue T, Ohtani K, Maehara S, Yamada M, Tsutsui H, Okunaka T, Kato H, Ikeda N. Tailor-made approach to photodynamic therapy in the treatment of cancer based on Bcl-2 photodamage. Int J Oncol. 2008;33:689–96.
103. Ichinose S, Usuda J, Hirata T, Inoue T, Ohtani K, Maehara S, Kubota M, Imai K, Tsunoda Y, Kuroiwa Y, Yamada K, Tsutsui H, Furukawa K, Okunaka T, Oleinick NL, Kato H. Lysosomal cathepsin initiates apoptosis, which is regulated by photodamage to Bcl-2 at mitochondria in photodynamic therapy using a novel photosensitizer, ATX-s10 (Na). Int J Oncol. 2006;29:349–55.
104. Caruso JA, Mathieu PA, Joiakim A, Leeson B, Kessel D, Sloane BF, Reiners JJ Jr. Differential susceptibilities of murine hepatoma 1c1c7 and Tao cells to the lysosomal photosensitizer NPe6: influence of aryl hydrocarbon receptor on lysosomal fragility and protease contents. Mol Pharmacol. 2004;4:1016–28.
105. Shaw P, Bovey R, Tardy S, Sahli R, Sordat B, Costa J. Induction of apoptosis by wild-type p53 in a human colon tumor-derived cell line. Proc Natl Acad Sci USA. 1992;89:4495–9.
106. Siognov RV, Haupt Y. The cellular response to p53: the decision between life and death. Oncogene. 1999;18:6145–57.
107. Fisher AM, Rucker N, Wong S, Gomer CJ. Differential photosensitivity in wild-type and mutant p53 human colon carcinoma cell lines. J Photochem Photobiol. 1998;42:104–7.
108. Fisher AMR, Danenberg K, Banerjee D, Bertino JR, Danenberg P, Gomer CJ. Increased photosensitivity in HL60 cells expressing wild-type p53. Photochem Photobiol. 1997;66:265–70.
109. Fisher AMR, Ferrario A, Rucker, N, Zhang S, Gomer CJ. Photodynamic therapy sensitivity is not altered in human tumor cells after abrogation of p53 function. Cancer Res. 1999;59:331–5.
110. Zawacka-Pankau J, Krachulec J, Grulkowski I, Bielawski KP, Selivanova G. The p53-mediated cytotoxicity of photodynamic therapy of cancer: recent advances. Toxicol Appl Pharmacol. 2008;232:487–97.
111. Kliukiene R, Maroziene A, Nivinskas H, Cenas N, Kirveliene V, Juodka B. The protective effects of dihydrolipoamide and glutathione against photodynamic damage by Al-phtalocyanine tetrasulfonate. Biochem Mol Biol Int. 1997;41:707–13.
112. Golab J, Nowis D, Skrzycki M, Czeczot H, Baranczyk-Kuzma A, Wilczynski GM, Makowski M, Mroz P, Kozar K, Kaminski R, Jalili A, Kopec' M, Grzela T, Jakobisiak M. Antitumor effects of photodynamic therapy are potentiated by 2-methoxyestradiol. A superoxide dismutase inhibitor. J Biol Chem. 2003;278:407–14.
113. Dolgachev V, Oberley LW, Huang TT, Kraniak JM, Tainsky MA, Hanada K, Separovic D. A role for manganese superoxide dismutase in apoptosis after photosensitization. Biochem Biophys Res Commun. 2005;332:411–7.

114. Oberdanner CB, Plaetzer K, Kiesslich T, Krammer B. Photodynamic treatment with fractionated light decreases production of reactive oxygen species and cytotoxicity in vitro via regeneration of glutathione. Photochem Photobiol. 2005;81:609–13.
115. Wang H, Qian S, Schafer F, Domann F, Oberley L, Buettner G. Phospholipid hydroperoxide glutathione peroxidase protects against singlet oxygen-induced cell damage of photodynamic therapy. Free Radic Biol Med. 2001;30:825–35.
116. Borst P, Evers R, Kool M, Wijnholds J. A family of drug transporters: the multidrug resistance-associated proteins. J Natl Cancer Inst. 2000;92:1295–1302.
117. Mikešová L, Mikeš J, Kovaľ J, Gyurászová K, Culka L, Vargová J, Valeková B, Fedoročko P. Conjunction of glutathione level, NAD(P)H/FAD redox status and hypericin content as a potential factor affecting colon cancer cell resistance to photodynamic therapy with hypericin. Photodiagnosis Photodyn Ther. 2013;10:470–83.
118. Aliya S, Reddanna P, Thyagaraju K. Does glutathione S-transferase Pi (GST-Pi) a marker protein for cancer? Mol Cell Biochem. 2003;253:319–27.
119. Kulbacka J, Chwilkowska A, Bar J, Pola A, Banas T, Gamian A, Saczko J. Oxidative alterations induced in vitro by the photodynamic reaction in doxorubicin-sensitive (LoVo) and -resistant (LoVoDX) colon adenocarcinoma cells. Exp Biol Med (Maywood). 2010;235:98–110.
120. Wright KE, MacRobert AJ, Phillips JB. Inhibition of specific cellular antioxidant pathways increases the sensitivity of neurons to meta-tetrahydroxyphenyl chlorin-mediated photodynamic therapy in a 3D co-culture model. Photochem Photobiol. 2012;88:1539–45.
121. Kimani SG, Phillips JB, Bruce JI, MacRobert AJ, Golding JP. Antioxidant inhibitors potentiate the cytotoxicity of photodynamic therapy. Photochem Photobiol. 2012;88:175–87.
122. Schlesinger M. Heat shock proteins. J Biol Chem 1990;265:12111–4.
123. Gomer CJ, Luna M, Ferrario A, Rucker N. Increased transcription and translation of heme oxygenase in Chinese hamster fibroblasts following photodynamic stress or Photofrin II incubation. Photochem Photobiol. 1991;53:275–9.
124. Curry PM, Levy JG. Stress protein expression in murine tumor cells following photodynamic therapy with benzoporphyrin derivative. Photochem Photobiol. 1993;58:374–9.
125. Gomer CJ, Ferrario A, Rucker N, Wong S, Lee AS. Glucose regulated protein induction and cellular resistance to oxidative stress mediated by porphyrin photosensitization. Cancer Res. 1991;51:6574–9.
126. Jozkowicz A, Was H, Dulak J. Heme oxygenase-1 in tumors: is it a false friend? Antioxid Redox Signal. 2007;9:2099–117.
127. Gomer C, Rucker N, Wong S. Porphyrin photosensitivity in cell lines expressing a heat-resistant phenotype. Cancer Res. 1990;50:5365–8.
128. Verwanger T, Sanovic R, Aberger F, Frischauf AM, Krammer B. Gene expression pattern following photodynamic treatment of the carcinoma cell line A-431 analysed by cDNA arrays. Int J Oncol. 2002;21:1353–9.
129. Hanlon J, Adams K, Rainbow A, Gupta R, Singh G. Induction of HSP60 by Photofrin-mediated photodynamic therapy. J Photochem Photobiol. 2001;64:55–61.
130. Wang H, Hanlon J, Rainbow A, Espiritu M, Singh G. Up-regulation of HSP27 plays a role in the resistance of human colon carcinoma HT29 cells to photooxidative stress. Photochem Photobiol. 2002;76:98–104.
131. Xue LY, He J, Oleinick NL. Rapid tyrosine phosphorylation of HS1 in the response of mouse lymphoma L5178Y-R cells to photodynamic treatment sensitized by the phthalocyanine Pc 4. Photochem Photobiol. 1997;66:105–13.
132. Nowis D, Legat M, Grzela T, Niderla J, Wilczek E, Wilczynski GM, Głodkowska E, Mrówka P, Issat T, Dulak J, Józkowicz A, Waś H, Adamek M, Wrzosek A, Nazarewski S, Makowski M, Stokłosa T, Jakóbisiak M, Gołab J. Heme oxygenase-1 protects tumor cells against photodynamic therapy-mediated cytotoxicity. Oncogene. 2006;25:3365–74.
133. Firczuk M, Gabrysiak M, Barankiewicz J, Domagala A, Nowis D, Kujawa M, Jankowska-Steifer E, Wachowska M, Glodkowska-Mrowka E, Korsak B, Winiarska M, Golab J. GRP78-targeting subtilase cytotoxin sensitizes cancer cells to photodynamic therapy. Cell Death Dis. 2013;4:e741.

134. Järveläinen H, Sainio A, Koulu M, Wight TN, Penttinen R. Extracellular matrix molecules: potential targets in pharmacotherapy. Pharmacol Rev. 2009;61:198–223.
135. Frantz C, Stewart K, Weaver V. The extracellular matrix at a glance. J Cell Sci. 2010;123:4195–200.
136. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell. 2002;110:673–87.
137. Rippa AL, Vorotelyak EA, Vasiliev AV, Terskikh VV. The role of integrins in the development and homeostasis of the epidermis and skin appendages. Acta Naturae. 2013;5:22–33.
138. Cianfriglia M, Yassen A, Tombesi M, Samoggia P, Barca S, Caserta M. Expression of lymphocyte homing receptor gene is lost in multi-drug-resistant variant of human lymphoblastoid CCRF-CEM cells. Int J Cancer. 1991;49:394–7.
139. Thompson E, Paik S, Brunner N, Sommers C, Zugmaier G, Clarke R, Shima T, Torri J, Donhaue S, Lippman M, Martin G, Dickson R. Association of increased basement membrane invasiveness with absence of estrogen receptor and expression of vimentin in human breast cancer cell lines. J Cell Physiol. 1992;150:534–44.
140. Hazlehurst LA, Dalton WS. Mechanisms associated with cell adhesion mediated drug resistance (CAM-DR) in hematopoietic malignancies. Cancer Metastasis Rev. 2001;20:43–50.
141. Schmidmaier R, Baumann P. ANTI-ADHESION evolves to a promising therapeutic concept in oncology. Curr Med Chem. 2008;15:978–90.
142. Cordes N, Meineke V. Cell adhesion-mediated radioresistance (CAM-RR): extracellular matrix-dependent improvement of cell survival in human tumor and normal cells in vitro. Strahlenther Onkol. 2003;179:337–44.
143. Cordes N. Overexpression of hyperactive integrin-linked kinase leads to increased cellular radiosensitivity. Cancer Res. 2004;64:5683–92.
144. Margaron P, Sorrenti R, Levy JG. Photodynamic therapy inhibits cell adhesion without altering integrin expression. Biochim Biophys Acta. 1997;1359:200–10.
145. Runnels JM, Chen N, Ortel B, Kato D, Hasan T. BPD-MA mediated photosensitization in vitro and in vivo: cellular adhesion and beta1 integrin expression in ovarian cancer cells. Br J Cancer. 1999;80:946–53.
146. Rousset N, Vonarx V, Eleouet S, Carre J, Kerninon E, Lajat Y, Patrice T. Effects of photodynamic therapy on adhesion molecules and metastasis. J Photochem Photobiol B. 1999;52:65–73.
147. Momma T, Hamblin MR, Wu HC, Hasan T. Photodynamic therapy of orthotopic prostate cancer with benzoporphyrin derivative: local control and distant metastasis. Cancer Res. 1998;58:5425–31.
148. Casas A, Di Venosa G, Vanzulli S, Perotti C, Mamome L, Rodriguez L, Simian M, Juarranz A, Pontiggia O, Hasan T, Batlle A. Decreased metastatic phenotype in cells resistant to aminolevulinic acid-photodynamic therapy. Cancer Lett. 2008;271:342–51.
149. Foultier M, Vonarx-Coinsmann V, Cordel S, Combre A, Patrice T. Modulation of colonic cancer cell adhesiveness by haematoporphyrin derivative photodynamic therapy. J Photochem Photobiol. 1994;23:9–17.
150. Vonarx V, Foultier M, de Brito X, Anasagasti L, Morlet L, Patrice T. Photodynamic therapy decreases cancer colonic cell adhesiveness and metastatic potential. Res Exp Med. 1995;195:101–16.
151. Overhaus M, Heckenkamp J, Kossodo S, Leszcynski D, La Muraglia G. Photodynamic therapy generates a matrix barrier to invasive vascular cell migration. Circ Res. 2000;86:334–40.
152. Lee C, Wu S, Chen L. Photosensitization by 3,3'-dihexyloxacarbocyanine iodide: specific disruption of microtubules and inactivation of organelle motility. Cancer Res. 1995;55:2063–9.
153. Ruoslahti E. Fibronectin and its integrin receptors in cancer (Review). Adv Cancer Res. 1999;76:1–20.
154. Sanovic R, Krammer B, Grumboeck S, Verwanger T. Time-resolved gene expression profiling of human squamous cell carcinoma cells during the apoptosis process induced by photodynamic treatment with hypericin. Int J Oncol. 2009;35:921–39.

155. Buytaert E, Matroule JY, Durinck S, Close P, Kocanova S, Vandenheede JR, de Witte PA, Piette J, Agostinis P. Molecular effectors and modulators of hypericin-mediated cell death in bladder cancer cells. Oncogene. 2008;27:1916–29.
156. Galaz S, Espada J, Stockert JC, Pacheco M, Sanz-Rodriguez F, Arranz R, Rello S, Canete M, Villanueva A, Esteller M, Juarranz A. Loss of E-cadherin mediated cell-cell adhesion as an early trigger of apoptosis induced by photodynamic treatment. J Cell Physiol. 2005;205:86–96.
157. Casas A, Sanz-Rodriguez F, Di Venosa G, Rodriguez L, Mamone L, Blázquez A, Jaén P, Batlle A, Stockert JC, Juarranz A. Disorganisation of cytoskeleton in cells resistant to photodynamic treatment with decreased metastatic phenotype. Cancer Lett. 2008;270:56–65.
158. Giese G, Schmidt J, Gilbert M, Albrecht R, Traub P. TPA induces apoptosis in MPC-11 mouse plasmacytoma cells grown in serum-free medium. Biol Cell. 1997;89:99–111.
159. Hashimoto M, Inoue S, Ogawa S, Conrad C, Muramatsu M, Shackelford D, Maliah E. Rapid fragmentation of vimentin in human skin fibroblasts exposed to tamoxifen: a possible involvement of caspase-3. Biochem Biophys Res Commun. 1998;247:401–6.
160. Belichenko I, Morishima N, Separovic D. Caspase-resistant vimentin suppressed apoptosis after photodynamic treatment with a silicon phthalocyanine in Jurkat cells. Arch Biochem Biophys. 2001;390:57–63.
161. Perry R, Matthews W, Mitchell J, Russo A, Evans S, Pass H. Sensitivity of different human lung cancer histologies to photodynamic therapy. Cancer Res. 1990;50:4272–6.
162. Dodson JW, Hay ED. Secretion of collagen by corneal epithelium. II. Effect of the underlying substratum on secretion and polymerisational epithelial cell products. J Exptl Zool. 1974;789:51–72.
163. Gospodarowicz D, Greenburg G, Birdwell CR. Determination of cellular shape by the extracellular matrix and its correlation with the control of cellular growth. Cancer Res. 1978;38:4155–71.
164. Brambilla E, Moro D, Gazzeri S, Brichon P, Nagy-Mignotte H, Morel F, Jacrot M, Brambilla C. Cytotoxic chemotherapy induces cell differentiation in small-cell lung carcinoma. J Clin Oncol. 1991;9:50–61.
165. Richter A, Kelly B, Chow J, Liu D, Towers G, Dolphin D, Levy J. Preliminary studies on a more effective phototoxic agent than hematoporphyrin. J Natl Cancer Inst. 1987;79:1327–2.
166. Porro A, Chrochemore C, Cambuli F, Iraci N, Contestabile A, Perini G. Nitric oxide control of MYCN expression and multi drug resistance genes in tumors of neural origin. Curr Pharm Des. 2010;16:431–9.
167. Esteban FJ, Horcajadas A, El-Rubaidi O, Luque-Barona R, Ibáñez G, García-Carriazo A, Segovia M, del Moral-Leal ML. Nitric oxide in malignant astrocytes. Rev Neurol. 2005;40:437–40.
168. Leung EL, Fraser M, Fiscus RR, Tsang BK. Cisplatin alters nitric oxide synthase levels in human ovarian cancer cells: involvement in p53 regulation and cisplatin resistance. Br J Cancer. 2008;98:1803–09.
169. Rubbo H, Radi R, Trujillo M, Kalyanaraman B, Barnes S, Freeman BA. Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. J Biol Chem. 1994;269:26066–75.
170. Niziolek M, Korytowski W, Girotti AW. Chain-breaking antioxidant and cytoprotective action of nitric oxide on photodynamically stressed tumor cells. Photochem Photobiol. 2003;78:262–70.
171. Fetz V, Bier C, Habtemichael N, Schuon R, Schweitzer A, Kunkel M, Engels K, Kovács AF, Schneider S, Mann W, Stauber RH, Knauer SK. Inducible NO synthase confers chemoresistance in head and neck cancer by modulating survivin. Int J Cancer. 2009;124:2033–41.
172. Kim YM, Bergonia H, Lancaster JR. Nitric oxide-induced autoprotection of isolated rat hepatocytes. FEBS Lett. 1995;374;228–32.
173. Joshi MS, Ponthier JL, Lancaster JR. Cellular antioxidant and prooxidant actions of nitric oxide. Free Radic Biol Med. 1999;27:1357–66.
174. Niziolek M, Korytowski W, Girotti AW. Nitric oxide-induced resistance to lethal photooxidative damage in a breast tumor cell line. Free Radic Biol Med. 2006;40:1323–31.

175. Niziolek-Kierecka M, Pilat A, Korytowski W, Girotti AW. Apoptosis-accommodating effect of nitric oxide in photodynamically stressed tumor cells. Photochem Photobiol. 2010;86:681–6.
176. Zareba M, Niziolek M, Korytowski W, Girotti AW. Merocyanine 540-sensitized photokilling of leukemia cells: role of post-irradiation chain peroxidation of plasma membrane lipids as revealed by nitric oxide protection. Biochim Biophys Acta. 2005;1722:51–9.
177. Di Venosa G Casas A Fukuda H Perotti C Batlle A. No cross-resistance between ALA-mediated photodynamic therapy and nitric oxide. Nitric Oxide. 2005;13:155–62.
178. Di Venosa G Perotti C Fukuda H Batlle A Casas A. Sensitivity to ALA-PDT of cell lines with different nitric oxide production and resistance to NO cytotoxicity. J Photochem Photobiol B. 2005;80:195–202.
179. Bhowmick R, Girotti AW. Cytoprotective Induction of nitric oxide synthase in a cellular model of 5-aminolevulinic acid-based photodynamic therapy. Free Radic Biol Med. 2010;48:1296–301.
180. Bhowmick R, Girotti AW. Cytoprotective signaling associated with nitric oxide upregulation in tumor cells subjected to photodynamic therapy-like oxidative stress. Free Radic Biol Med. 2013;57:39–48.
181. Vaupel P, Harrison L. Tumor hypoxia: causative factors, compensatory mechanisms, and cellular response. Oncologist. 2004;9:4–9.
182. Ferrario A, von Tiehl KF, Rucker N, Schwarz MA, Gill PS, Gomer CJ. Antiangiogenic treatment enhances photodynamic therapy responsiveness in a mouse mammary carcinoma. Cancer Res. 2000;60:4066–9.
183. Okunaka T, Usuda J, Ichinose S, Hirata H, Ohtani K, Maehara S, Inoue T, Imai K, Kubota M, Tsunoda Y, Kuroiwa Y, Tsutsui H, Furukawa K, Nishio K, Kato H. A possible relationship between the anti-cancer potency of photodynamic therapy using the novel photosensitizer ATX-s10-Na(II) and expression of the vascular endothelial growth factor in vivo. Oncol Rep. 2007;18:679–83.
184. Askari JA, Buckley PA, Mould AP, Humphries MJ. Linking integrin conformation to function. J Cell Sci. 2009;122:165–70.
185. Reyal J. Arterial flow focalization could increase tissue oxygen partial pressure, or trigger endothelial shear stress—a new concept to overcome cancer hypoxia-induced radiotherapy resistance, or stimulate liver regeneration during fulminant hepatitis. Med Hypotheses. 2009;74:301–8.
186. Boyer M. Bioreductive agents: a clinical update. Oncol Res. 1997;9:391–5.
187. Patterson A, Williams K, Cowen R, Jaffar M, Telfer B, Saunders M, Airley R, Honess D, van der Kogel A, Wolf C, Stratford U. Oxygen-sensitive enzyme-prodrug gene therapy for the eradication of radiation-resistant solid tumors. Gene Ther. 2002;9:946–54.
188. Aemany-Ribes M, García-Díaz M, Busom M, Nonell S, Semino CE. Toward a 3D cellular model for studying in vitro the outcome of photodynamic treatments: accounting for the effects of tissue complexity. Tissue Eng Part A. 2013;19:1665–74.