

Chapter 2

Mechanisms of Resistance to Photodynamic Therapy: An Update

Adriana Casas, Christian Perotti, Gabriela Di Venosa and Alcira Batlle

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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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-hexyloxyethyl)-2-devinyl pyropheophorbide-a
iNOS	inducible NO synthase
L-NAME	NG-Nitro-L-arginine methyl ester
LOX	Lipoxygenase
MAPK	Mitogen-activated protein kinase
MDR	Multidrug resistance
MRP1	Multidrug resistant associated protein 1
m-THPC	5,10,15,20-tetra(<i>meta</i> -hydroxyphenyl) 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 pheophorbide 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 endocytosed 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-hexyloxyethyl)-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 phthalocyanine (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 ~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 ABCG2-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, ≥ 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

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