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Photodynamic therapy of cancer — Challenges of multidrug resistance

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Photodynamic therapy (PDT) of cancer is a two-step drug-device combination modality, which involves the topical or systemic administration of a photosensitizer followed by light illumination of cancer site. In the presence of oxygen molecules, the light illumination of photosensitizer (PS)

can lead to the generation of cytotoxic reactive oxygen species (ROS) and consequently destroy cancer. Similar to many other anticancer therapies, PDT is also subject to intrinsic cancer resistance mediated by multidrug resistance (MDR) mechanisms. This paper will review the recent progress in understanding the interaction between MDR transporters and PS uptake. The strategies that can be used in a clinical setting to overcome or bypass MDR will also be discussed.

Keywords: Photodynamic therapy; photosensitizer; multidrug resistance; cancer.

1. Introduction

Photodynamic therapy (PDT) of cancer involves the administration of a photosensitizer (PS) followed by illumination of the cancer site with visible light. This process can lead to generation of reactive oxygen species (e.g., singlet oxygen) in the presence of oxygen molecules *via* photon-induced energy and/or electron transfer. PDT-mediated oxidation can cause local cytotoxicity leading to cancer cell death through apoptosis and/or necrosis pathways. Unlike other oxidant-based cancer therapies, in addition to the rapid direct oxidation-driven cytotoxic effects on cancer cells, PDT-induced damage to the tumor vasculature, acute inflammatory reaction and systemic immunity also play significant roles in the anticancer effectiveness of PDT.[1]

As a clinically approved and minimally invasive therapeutic modality, PDT has been used for curative or palliative management of various premalignant (e.g., actinic keratoses, Barrett's Esophagus) and malignant diseases (e.g., obstructive lung cancer and esophageal cancer) worldwide.[2,3] Although it is generally believed that cancer has no significant resistance toward PDT and can be ultimately ablated through maximizing PDT drug and/or light dose, a better understanding of cancer biology suggests that similar to many other cancer therapies, PDT is inevitably subject to intrinsic cancer resistance at the cellular and molecular level via drug efflux, hypoxia, levels of pigmentation and damage reversal mechanisms. Serious considerations have to be taken to overcome these defense mechanisms in order to make anticancer PDT more effective and acceptable by mainstream medicine.

This review paper will primarily discuss the challenges of cancer resistance involved in the cellular uptake of PS. The strategies that can be used in the clinical setting to overcome this particular type of resistance will also be discussed.

2. Photosensitizer

2.1. *Classification of PS*

PS is one of three critical elements in PDT (i.e., PS, light and oxygen). The majority of PSs possess a heterocyclic ring structure (e.g., tetrapyrrole ring) similar to that of hematoporphyrin or chlorophyll. In general, they can be divided into three broad families: (i) porphyrin-based PS (e.g., Photofrin or Porfimer Sodium, benzoporphyrin derivative (BPD), hematoporphyrin monomethyl ether (HMME, or Hemoporfin)), (ii) chlorophyll-based PS (e.g., chlorins, bacteriochlorins, purpurins) and (iii) dye-based PS (e.g., phtalocyanine, napthalocyanine). Both hematoporphyrin and chlorine PSs are also classified as porphyrins. Many PSs possess distinct and strong fluorescence that can be used for their *in vivo* detection and quantification, which provides a unique tool for photodynamic diagnosis and theranostics.[4]

Traditionally, the porphyrins and those PSs developed in the 1970s and early 1980s are called the first generation PSs (e.g., Photofrin). Porphyrin derivatives or synthetics of known chemical structures made since the late 1980s are called the second generation PSs (e.g., m-tetrahydroxyphenylchlorin (mTHPC), BPD-MA, HMME, hexyloxyethyl pyropheophorbide-a (HPPH)). 5-Aminolaevulinic acid (ALA) and its ester derivatives are also often called PS but they are the prodrug of protoporphyrin IX (PpIX).[5] The third generation PSs generally refers to the modifications such as biologic conjugates (e.g., antibody conjugate) and built-in photo quenching or bleaching capability. Target-specific PDT refers to the use of the antibody- or antisense-conjugated PSs, which combines the specificity to an over-expressed cellular marker with the phototoxic properties of the conjugated PDT PS. The targeted cellular marker can be a cancer-associated or noncancer-associated marker. The conjugation may or may not necessarily enhance the internalization process, although the internalization might enhance PDT-induced cytotoxicity.

2.2. *Mode of actions*

PSs are currently administrated systemically (mainly intravenously) or topically (mainly for prodrugs such as heme precursor) in clinical settings. The cancer-localizing properties of PS might include the preponderance of leaky and tortuous blood vessels due to neovascularization and the absence of lymphatic drainage known as the enhanced permeability and retention effect of PS in tumor tissues. Some of the most effective PSs bind preferentially to low-density lipoprotein (LDL), suggesting that upregulated LDL receptors in cancer cells could be an important factor in PS uptake.[1] PSs can be covalently attached to various biomolecules that have some affinity for neoplasia or to receptors expressed on cancer cells and result in unique subcellular localization patterns. In general, water soluble porphyrin ethers (e.g., Photofrin) have variable localization patterns mostly associated with lipid membranes. Mono-L-aspartyl chlorin e6 (NPe6, talaporfin) targets the lysosomes. BPD targets the mitochondria. mTHPC can target the mitochondria, endoplasmic reticulum (ER), or both. Phthalocyanine Pc4 has a broad spectrum of affinity to different organelles.[6] Nevertheless, it should be noted that specific patterns of cellular localization may vary among different cell types and therefore trigger different photocytotoxicity. The presence of competitive binding agents can also influence the subcellular localization and/or binding of PSs.[7] As one can expect, the presence of prooxidant factors in cancer cells might scavenge PDT-induced oxygen species and therefore have a negative effect on PDT efficacy.[8]

Great variations in PS uptake can be found between individual cell lines, resulting in even more pronounced differences in photocytotoxicity. It is important to be able to predict PS uptake profiles in the clinical setting in order to adjust the dose for effective and complete cancer elimination. Meanwhile, one should be aware that at equivalent cellular PS levels, there are many other factors (e.g., level of oxygenation, presence of antioxidant) that might affect the sensitivity of cancer cells to PDT.

3. MDR and PS Uptake

3.1. *MDR transporters*

Multidrug resistance (MDR) is a phenomenon where resistance to one anticancer chemotherapy drug is accompanied by resistance to drugs with different chemical structures and mechanism of action.[9] MDR is often attributed to the over-expression of certain members of ATP-binding cassette (ABC) transporter proteins (also known as efflux pumps) including p-glycoprotein (P-gp/ABCB1/MDR1), multidrug resistance proteins (MRPs, e.g., MRP1-9), and breast cancer resistance proteins (BCRP/ABCG2/MXR/ABCP).[10] ABC transporters form one of the largest protein superfamilies encoded in the human genome, and more than 48 human ABC protein genes have been identified.

3.2. *Interaction of MDR and PS*

Early studies in the 1990's showed that PDT-resistant variants obtained from multiple PDT treatments did not exhibit MDR phenotype nor did they have altered the uptake properties of porphyrin-based PS.[11,12] PDT-resistant variants did not display a broad cross-resistance to different types of PSs, suggesting that the mechanism of PDT resistance may, to some extent, depend upon the physical nature of the PS molecule.[13] It was generally believed then that chemotherapy-resistance or radiotherapy-resistance was not significantly cross-resistant to porphyrin-based PDT nor did PDT induce resistance to chemotherapy or radiotherapy.

Some PSs might even act as a MDR reverser, e.g., methylene blue (MB) on P-gp.[14] Early studies also suggested that MDR variants could be sensitive to PDT which supports the use of PDT for MDR cancer.[15–19] Milla *et al.* showed that PDT-resistant squamous carcinoma cells had a more fibroblastic morphology, higher number of stress fibers, more expression of cell-substrate adhesion proteins and higher expression of phospho-survivin but few differences in intracellular PpIX content after incubation with ALA methyl derivative.[20] Topical ALA PDT has been successfully used for some resistant cases of cutaneous T-cell lymphoma.[21] Chu and Yow showed that hexyl ALA-mediated PDT could significantly provoke an up-regulation of phosphorylated p38MAPK and c-Jun N-terminal kinase (JNK) proteins in P-gp expressing doxorubicin-resistant human uterine sarcoma cells (MES-SA/Dx5).[22] Interestingly, a recent study suggests that pheophorbide-a mediated PDT could inhibit the MDR activity by down-regulating the expression of P-gp *via* JNK activation.[23]

However, the heterogeneity of PS uptake has been demonstrated in various *in vitro* and *in vivo*

models.[24,25] The mechanisms of resistance to PDT ascribed to the PS may be shared with the general mechanisms of MDR, and are related to altered PS uptake and efflux rates or altered intracellular trafficking within cancer cells.[26]

3.3. *P-gp and PS uptake*

In the mid-1990's, Luna *et al.* demonstrated that certain cellular receptors (e.g., alpha-2 macroglobulin receptor/low density lipoprotein receptor-related protein) could modulate PS uptake and affect PDT sensitivity of targeted cells.[27] For the first time, Purkiss *et al.* reported that the P-gp export mechanism may have an effect on the cytotoxicity of PDT by reducing the concentration of hematoporphyrin derivative (HpD) within human colorectal cancer cells (HRT 18) since P-gp mediated resistance to PDT could be reversed through modulation with verapamil (an antagonist of P-gp).[28] An *in vitro* study demonstrated that the addition of verapamil could increase the intracellular levels of PSD-007 (a mixture of hematoporphy derivative) and DNA content in colon cancer cells, meanwhile decreasing S and G1 phase cells.[29] Although this is contradictory to early studies showing that over-expression of P-gp in a mouse fibroblast cell line (3T3 cells) had no significant effect on the cellular concentration of chloroaluminum tetrasulfonate phthalocyanine (AlSPc) nor did over-expression of P-gp in human breast adenocarcinoma cells (MCF-7 cells) affect chlorin e6 accumulation.[30,31] Early studies also suggested that the intra and extracellular PpIX accumulation mediated by ALA were not subjected to the level of P-gp expression,[32] but the rapid efflux of PpIX had also been demonstrated in DT-resistant variants.[7] Savitskiy *et al.* showed that the specific cellular protein P-gp 170 did not appear to alter the intracellular accumulation of chlorins.[33] Later Saczko *et al.* showed that P-gp appeared to play a role in the intracellular accumulation of Photofrin but not hypericin in doxorubicin-resistant human colon cancer cell lines (LoVo cells).[34] Horibe *et al.* demonstrated that cisplatin resistance in A549 cells that had high level of P-gp mRNA had no significant influence on accumulation and photodynamic activity of chlorin e6.[35] Most transporters have transmembrane domains (TMD). P-gp are confined to membrane loci associated with the transporter and it was believed that it might have little effect on the migration of cytotoxic photo-products.[36] Nevertheless, the influence of P-gp on PS uptake in cancer cells remains inconclusive.

3.4. *BCRP and PS uptake*

In addition to early interest in the effect of P-gp expression and efflux mechanism on PS uptake, for the first time Robey *et al.* demonstrated in the early 2000's that PSs with similar structure to that of pheophorbide-a were a substrate of BCRP but not to other major drug efflux transporters such as P-gp or MRP1.[37,38] They reported that BCRP-transfected human embryonic kidney cells (HEK-293 cells) were 11-fold, 30-fold, 4-fold, and >7-fold resistant to PDT mediated with pheophorbide a, pyropheophorbide a methyl ester, chlorin e6 and ALA, respectively. BCRP, a member of the phase III system of xenobiotic metabolism, is responsible for protecting the body from toxic xenobiotics and for removing toxic metabolites, including the transport of porphyrin and chlorophyll metabolites. Liu *et al.* demonstrated that the use of tyrosine kinase inhibitors (e.g., imatinib mesylate) can block the function of BCRP and increase accumulation of HPPH, PpIX and BPD-MA from 1.3- to 6-fold in BCRP+ cells and consequently enhance PDT efficacy in RIF-1 tumor model.[39] Jendzelovský *et al.* showed that Proadifen, an inhibitor of cytochrome P450 enzymes, could affect the function of BCRP and MRP1 leading to increased hypericin content in colon cancer cells (HT-29 cells).[40] BCRP-mediated PpIX efflux was also a major factor that prevented PpIX accumulation in human urothelial carcinoma cells (T24 cells).[41] Bebes *et al.* showed that the PpIX extrusion ability of keratinocytes (HaCaT cells) was correlated with their BCRP expression which was higher in proliferating cells than in differentiated cells.[42] The specific inhibition of BCRP enhanced the sensitivity of keratinocytes to ALA PDT which improved the topical PDT of skin lesions. In addition to keratinocytes, it also enhanced the sensitivity of human esophagus cells (OE19 adenocarcinoma) and bladder cells (HT1197 carcinoma) to ALA/MAL PDT.[43] Along with BCRP, peptide transporter PEPT1 has been identified as an ALA influx transporter and participates in the regulation of intracellular PpIX levels in human gastric cancer cells.[44]

A large number of single nucleotide polymorphisms (SNP) were identified for a variety of drug

transporters, which provides a useful means to determine the relationship between nonsynonymous polymorphisms and the substrate specificity of drug transporter proteins. Based on SNP data, Tamura *et al.* demonstrated in insect *Spodoptera frugiperda* Sf9 cells that the amino acids at position 431, 441 and 489 located in TMD were critically involved in substrate recognition and/or transport of drugs.[45] More specifically, the S441N variant of BCRP completely lost transport activity for both hematoporphyrin and methotrexate but the F431L and F489L variants maintained hematoporphyrin transport but lost the activity of methotrexate transport. Later they showed that Flp-In-293 cells containing S441N and F489L variants exhibited high levels of both cellularly accumulated pheophorbide-a and photosensitivity. The accumulation of PpIX from ALA and pheophorbide-a in the cytoplasm compartment was maintained at low levels in Flp-In-293 cells expressing ABCG2 WT, V12M or Q141K. However, in the presence of BCRP inhibitor imatinib or novobiocin, those cells became sensitive to light.[45,46] They further demonstrated that the planar structure of inhibitors was an important factor for interactions with the active site of BCRP. These results suggested that certain genetic polymorphisms and/or inhibition of BCRP could enhance porphyrin-mediated photosensitivity. The over-expression of BCRP in glioma stem-like cells (GSC) isolated from U251 glioma cells can result in efflux of Photofrin, which can be reversed by a pretreatment of GSC with a specific BCRP inhibitor fumitremorgin C (FTC).[47] The DNA damage reversal mechanisms may have important functions in Photofrin PDT resistance through the activation of alkylation repair homologue 2 by tumor protein TP53 in glioma cells.[48]

The PS selectivity of BCRP was still unclear at that time. Usuda *et al.* demonstrated that BCRP-overexpressing human epidermoid carcinoma cells (A431 cells) were more resistant to Photofrin-PDT and FTC could reverse such resistance. However, the cell line did not show cross-resistance toward NPe6.[49] What was more significant was that they further examined 81 tumor specimens obtained from patients with centrally located early lung cancers that underwent PDT treatment. All specimens were BCRP-positive. The expression of BCRP significantly affected the efficacy of Photofrin-PDT in cancer lesions $\geq$10 mm in diameter. On the other hand, NPe6-PDT exhibited a strong antitumor effect, regardless of the expression status of BCRP in the lung cancers. They suggest that Photofrin may be a substrate of BCRP and be pumped out from cancer cells, therefore, the PS selectivity of BCRP may be a molecular determinant of the outcome of PDT. This translational study represents a milestone work in addressing the profound impact of cancer MDR on PS selectivity and PDT outcome.

PSs without substitutions including pyropheophorbides (e.g., HPPH) and purpurinimides are general substrates for BCRP. Morgan *et al.* demonstrated in BCRP-expressing HEK-293 cells that carbohydrate groups conjugated at positions 8, 12, 13 and 17 but not at position 3 could abrogate BCRP affinity regardless of structure or linking moiety.[50] Yet, they showed that in the murine mammary tumor (4T1) HPPH but not the galactose conjugate of HPPH selectively preserved a small ABCG2-expressing side population (SP) which is believed to be primarily responsible for tumor regrowth. A PDT-resistant SP may be responsible for recurrences observed both preclinically and clinically. The SP could be targeted by addition of imatinib mesylate and ultimately preventing PS efflux. Tracy *et al.* demonstrated in tumor/stroma co-cultures derived from lung cancers that HPPH was lost from fibroblastic cells more rapidly than from epithelial cells, even under low BCRP expression, facilitating selective eradication by PDT of epithelial over fibroblastic cells.[51] Enhanced BCRP expression led to the selective PDT survival of tumor cells in tumor/stroma co-cultures. This survival pattern was reversible through no-substrate HPPH derivatives or imatinib mesylate. They concluded that PS retention, not differences in subcellular distribution or cell signaling responses, was determining cell type selective death by PDT. Therefore, up-front knowledge of cancer characteristics, specifically MDR status, could be helpful in individualizing PDT treatment design.

4. Possible Strategies in Clinical Setting

4.1. *Inhibition of MDR transporters*

To prevent MDR-mediated resistance, in addition to utilizing nonsubstrate PS and various PS conjugates, administering an MDR inhibitor alongside a substrate PS is another straightforward strategy to reduce the PS efflux rate in MRP expressing cells. Although numerous *in vitro* and *in vivo* studies show that the co-administration of a MRP inhibitor

could reverse the effect of certain MRP transporters on PS accumulation, such combination has not yet been tested in clinic.

The utilization of MRP inhibitor (or modulator or antagonist) in the treatment of solid tumor is under clinical investigation worldwide. Preliminary data suggest that for P-gp, a single dose of verapamil 120 mg or 80 mg three times daily (total daily doses of 240 mg) for 6 days could improve bioavailability of P-gp substrates or chemotherapy drugs.[52,53] It is noteworthy that clinical trials indicated that the P-gp-modulating agent Valspodar did not improve the treatment outcome of refractory multiple myeloma and P-gp inhibitors may not be a practical solution to MDR. Ceramide, the central molecule of sphingolipid metabolism, generally mediates antiproliferative and proapoptotic functions. Changes of the bioactive sphingolipid ceramide in various types of cancer cells have been observed in response to PDT. Korbelik *et al.* demonstrated that combining ceramide analog treatment with PDT could enhance intracellular calcium release in cancer cells and strongly promote apoptosis after PDT treatment.[54] Since the mechanism underlying the drug resistance which develops with increased glucosylceramide expression is associated with P-gp overexpression,[55] it can be expected that combining P-gp inhibitors with PDT might enhance the ability to generate intracellular ceramide and amplify apoptotic death. Nevertheless, Wagner *et al.* demonstrate that the third-generation P-gp modulator tariquidar could inhibit P-gp function at the human blood–brain barrier (BBB), which might be a useful approach for PDT of brain tumor.[56] Recently, Sun *et al.* demonstrated that pretreatment of human glioma cells with Gefitinib, a BCRP inhibitor, could enhance intracellular accumulation of PpIX through the inhibition of BCRP expression and BCRP-mediated PpIX efflux, ultimately improving the effectiveness of ALA-PDT.[57]

In addition to chronic myelogenous leukemia, the first indication for imatinib mesylate (Gleevec or Glivec) for the treatment of solid tumor was approved by the US Food and Drug Administration (FDA) in 2001. For advanced tumor, the recommended dose of imatinib mesylate as a molecular targeted cancer drug is 400 or 600 mg daily. However, chronic exposure to imatinib was shown to result in upregulation of P-pg and BCRP transporters in Caco-2 cells, but this phenomenon was not reproducible in the hepatic and intestinal compartments in mice.[58,59]

These preclinical and clinical studies suggest that the co-administration of a MRP inhibitor and PS might be a feasible strategy for PDT. Preclinical studies show that co-administration of imatinib mesylate and PS could indeed inhibit PS efflux although their combination in clinical setting still needs to be optimized and validated. Nevertheless, it should be noted that the intervals between the administration of inhibitor and chemotherapy drugs (or PS) is critical for the inhibitor to be effective. Dosing and scheduling of co-administration of inhibitor and PS should be carefully explored due to different pharmacokinetics of PS and inhibitor. Moreover, the use of inhibitor might increase systemic toxicity of chemotherapy. This could be a concern for PS since the increase of PS influx and peripheral PS accumulation might alter the skin photosensitization.

4.2. *Antivascular PDT*

Cancer treatment can be exerted by targeting both cancer cells and the vasculature supplying solid tumors. Antivascular PDT or vascular-targeting PDT (VTP or vPDT) represents the recent progress in PDT that can meet the paradigm shift in cancer treatment. vPDT is characterized by a short drug to light interval (DLI), typically 0–30 min after the completion of intravenous (iv) injection of PS. The PS used in vPDT should have fast clearance and therefore might not selectively accumulate in cancer cells. In vPDT, light irradiation takes place while the PSs are still circulating in the vascular compartment and, therefore, cause vascular damage and lead to thrombosis and micro-vessel occlusion.[60] vPDT has been used primarily for the management of the neovascularization lesions (e.g., wet age-related macular degeneration, AMD) and cutaneous capillary malformations (e.g., port wine stain birthmarks, PWS).[61,62] For MDR cancers, vPDT can target nonmalignant vascular network — the lifeline of cancer and therefore bypass MDR transporters and offer a novel approach to treat MDR expressing solid tumors. Although vPDT might change the traditional criteria of PS selection, longer wavelength and rapid clearance might be the key criteria for designing a PS for antivascular PDT.

Pd-bacteriochlorophyll based PSs have a high extinction coefficient in the near-infrared (IR) spectrum and rapid clearance from the blood

circulation and skin after iv injection. Preise *et al.* showed that P-gp expressing human HT29/MDR colon carcinoma cells were resistant *in vitro* to PDT medicated with Pd-bacteriopheophorbide (TOOKAD, also known as WST09), however, the vPDT with iv injection of TOOKAD and immediate light irradiation (0 min DLI) induced tumor necrosis with equal efficacy in HT29/MDR-derived xenografts and their wild-type counterparts.[63] These results are ascribed to the rapid antivascular effects of vPDT, suggesting that MDR cancers can be successfully eradicated by indirect approaches that bypass their inherent drug resistance. Moreover, targeting tumor vessels and angiogenesis might reduce the risk of metastasis.[64]

Permanent occlusion of feeding arteries and draining veins in solid tumor have been demonstrated in vPDT of mouse model.[65] vPDT mediated with Pd-bacteriochlorophyll derivatives (e.g., Tookad, and WST11) and interstitial irradiation has been investigated for the curative or palliative treatment of prostate cancer.[66–68] The massive shutdown of pathological and normal vessels in the tumor can deprive the supply of oxygen and nutrients and subsequently achieve tumor ablation. To ablate a bulky solid tumor it might require combining both cellular-targeting and vascular-targeting approaches. Co-administration of anti-angiogenic agent (e.g., inhibitor of pro-angiogenic factor, endogenous inhibitor) and use of nanocarriers consisting of vasculature targeting agent (e.g., NRP-1 peptide) and PS represent some new developments in targeted therapy.[69]

4.3. *Photochemical internalization*

Photochemical internalization (PCI) is a novel site-specific drug and gene delivery method developed to improve the intracellular release of macromolecules and hydrophilic chemotherapeutic agents from endosomes and lysosomes.[70] PCI is based on the combination of endosomal and lysosomal localizing amphiphilic PSs and light, therefore it could be considered as a form of intracellular PDT with a primary goal of time- and space-controlled and light-triggered drug delivery. After activating PS by light, photodynamic reactions result in destruction of endocytic vesicle membranes and subsequently release the entrapped drugs into the cytosol of targeted cells. Although PCI might reverse or bypass the MDR phenotype by endo-lysosomal release of the MDR substrate drug, PCI of macromolecular therapeutic agents that are not targets of MDR transporters represents another therapeutic strategy to treat MDR cancer.[71,72] Taking advantages of nanocarriers might further extend the efficacy of PDT and PCI.[73–75] PCI of numerous macromolecules has been demonstrated *in vitro* and *in vivo*. Disulfonated tetraphenyl chlorin ($TPCS_{2a}$) is found to be a clinically suitable PCI PS for photochemical activation of molecules that do not readily penetrate the cellular plasma membrane. It is currently subject to a first clinical trial in patients with various cancers. Preliminary results suggest that PCI seems to be a promising treatment modality for MDR cancer. PCI may exert direct cytotoxic effects on endothelial cells, which lays a foundation for utilizing the PCI technology as an antivascular strategy to ablate tumors.[76]

4.4. *Intratumoral injection*

A systemic administration of certain PSs can cause prolonged skin photosensitization and result in poor tumor selectivity. These drawbacks might be overcome by intratumoral injection of PS. For small and localized cancers, there is still a need to explore intratumoral delivery approaches, this is particularly true since many cancers are detected at an early stage at a small size.

Early studies suggest that intratumoral injection is effective for tumor of small sizes (<8 mm in diameter).[77–79] The effectiveness and safety of intratumoral injection of PS can be affected by drug formulation, injection volume, velocity and site. The use of lipid-based PS (e.g., Foscan) and nanocarriers might improve the PS distribution and retention in targeted tumor.[80]

An effective control of cancer might require the selective destruction of parenchymal and/or stromal tissue. It is well known that the generation of a reactive stroma environment can promote tumorigenesis. Fibroblast-activation protein (FAP) is a membrane-bound serine protease that is expressed on the surface of reactive stromal fibroblasts present within the majority of human epithelial cancers but is not expressed by normal tissues. Therefore, FAP represents a potential pan-tumor target whose enzymatic activity can be exploited for the intratumoral activation of prodrugs and protoxins.[81] This

represents a paradigm shift in cancer therapy and inspires people to develop PS suitable for intratumoral injection that can bypass MDR and generate a high PS concentration inside tumor stromal tissues. Lo *et al.* developed a novel FAP-triggered PDT beacon which could induce significant photocytotoxicity in FAP-expressing cells and be activated in FAP-expressing stromal fibroblasts *in vivo*.[82] Although many PS-conjugates are unsuitable for systemic administration for many reasons (e.g., systemic toxicity, high cost), they may be an ideal candidate for intratumoral delivery.

4.5. *Nanodelivery*

Drug delivery is a key determinant of drug efficacy in cancer chemotherapy. Because of unique physicochemical properties of nanomaterials, such as small size, large surface area to mass ratio and high reactivity, nanocarrier-based approaches have shown great promise for carrying, protecting and delivering potential therapeutic molecules with diverse physiological properties. Current nanotechnology is revolutionizing drug delivery by improving pharmacokinetics, biodistribution, specificity and molecular targeting of cancer therapeutics. Nanocarrier-based approaches not only can circumvent limitations in the delivery of cancer therapeutics, related to their poor aqueous solubility and toxicity issues with conventional vehicles, the use of nanocarriers (e.g., liposomes, nanoemulsions, nanoparticles, carbon nanotubes) can also overcome MDR in cancer therapy.[83] Some of nanopreparations have advanced to clinical trials.

It is highly likely that nanotechnology will modify and alter both the basic science and clinical applications of PDT.[84,85] Noticeably, most of the current studies are aimed at either improving existing formulations of clinically approved PS or focused on the development of targeted delivery vehicles.[86] Some of the PS currently used in clinics are in fact nanosized materials, e.g., liposomal formulations (e.g., Foscan, Visudyne), which have shown improved PS distribution and retention in targeted tumor.[80] Actively targeted liposomes can be developed by conjugating ligands (e.g., glycoproteins, peptides, oligonucleotide aptamers, antibodies) to the liposomal surface which allow specific targeting to certain cancer cells.

In addition to the potentials of nanodelivery of PS in vasculature targeting and PCI approaches,[69,73–75] the combination nanocarrier for dual modalities has also been used to overcome drug resistance. Khdair *et al.* showed that a combination of doxorubicin and MB bound to Aerosol OT alginate nanoparticles had significant therapeutic potential against tumors expressing P-gp.[87]

The most advanced nanocarrier systems combining disease diagnosis with therapy (theranostics) is also noteworthy. Due to the dual functions of fluorescence and photosensitization, PS is a good candidate for developing cancer theranostics.[88]

4.6. *Enhancing antitumor immunity*

Drug resistance strongly argue for innovative strategies to treat and manage cancer. Stimulating the power of cancer patient's own immune defense is a highly attractive strategy to complement the activity of standard chemotherapy. Moreover, several immunotherapy approaches could be used to combat cancer MDR. For instance, direct immune attack against MDR transporters, using MDR as an immune target to deliver cytotoxic agents, conditional immunotoxins expressed under MDR control, and modulating immunogenic potential of some cytotoxic agents.[89]

Preclinical studies have shown that PDT could enhance local and systemic antitumor immunity and increased expression of proinflammatory cytokines play a key role in initiating specific cellular and humoral antitumor immunity. The implications of PDT-induced antitumor immunity and efficacious PDT-generated vaccines provide a possibility for using PDT in the treatment of metastatic disease and as an adjuvant in combination with other modalities for treating MDR cancers.[1,90,91] On the other hand, the presence of an intact adaptive immune system could benefit the long-term efficacy of antitumor PDT since both the direct cancer cell killing and the control of cancer cells revival after treatment are equally crucial.

Although antitumor immunity is able to specifically target cancer cells, the existence of a variety of immune escape mechanisms can be involved in minimizing the overall effectiveness of cancer therapy. Therefore, the elimination of immunosuppressive activities in tumor microenvironment is another attractive strategy for enhancing the effectiveness of cancer immunotherapy. Immune-suppressive cells include a heterogeneous population of immature myeloid cells expanded systemically as a

consequence of a profound tumor-associated pro-inflammatory milieu. Recently, Barth *et al.* demonstrated in a mouse model that PDT might overcome immunosuppressive cells via the regulation of immature myeloid cells and the inflammatory milieu critical to their expansion during tumor progression.[92] Reginato *et al.* showed that the depletion of T-regulatory cells could potentiate PDT-mediated immunity.[93]

5. Conclusive Remarks

PS is a critical element in PDT. Although to a certain extent the quantity and location of PS can predict the nature of photodynamic reactions and determine the consequence of anticancer effect, it should be aware that at equivalent cellular PS levels, there are many other factors that might affect the sensitivity as well as phototoxicity of cancer cells to PDT. Mounting evidence suggests that many PSs are substrates of MDR transports and PS efflux mediated by P-gp and BCRP can negatively affect anticancer efficacy. Therefore, the screening of cancer MDR profile can be helpful in individualized PS selection and PDT treatment design.

To meet this significant paradigm shift and prevent MDR mediated resistance, in addition to utilizing nonsubstrate PS or PS conjugates, administering an MDR inhibitor alongside a substrate PS is a feasible strategy to reduce the PS efflux in MDR expressing cells. However, dosing and scheduling of co-administration of MDR inhibitor and PS are yet to be investigated since PS and inhibitor could have different pharmacokinetics.

Much progress has been seen in both basic research and clinical application in recent years. The majority of approved PDT clinical protocols have primarily been used for the treatment of superficial lesions of both malignant and nonmalignant diseases. The implication of antivascular PDT, PCI, nanodelivery and immunotherapy in bypassing the MDR transports represents novel approaches in anticancer PDT. It can be expected that in conjunction with PS of longer excitation wavelengths, these approaches might provide an effective alternative for the treatment of deep-seated tumors.

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