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PERSPECTIVE

Targeted photodynamic therapy – a promising strategy of tumor treatment

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Targeted therapy is a new promising therapeutic strategy, created to overcome growing problems of contemporary medicine, such as drug toxicity and drug resistance. An emerging modality of this approach is targeted photodynamic therapy (TPDT) with the main aim of improving delivery of photosensitizer to cancer tissue and at the same time enhancing specificity and efficiency of PDT. Depending on the mechanism of targeting, we can divide the strategies of TPDT into "passive", "active" and "activatable", where in the latter case the photosensitizer is activated only in the target tissue. In this review, contemporary strategies of TPDT are described, including new innovative concepts, such as targeting assisted by peptides and aptamers, multifunctional nanoplatforms with navigation by magnetic field or "photodynamic molecular beacons" activatable by enzymes and nucleic acid. The imperative of introducing a new paradigm of PDT, focused on the concepts of heterogeneity and dynamic state of tumor, is also called for.

1. Introduction

Photodynamic therapy (PDT) is a minimally invasive method that destroys target cells in the presence of oxygen when light irradiates a photosensitizer, generating reactive oxygen species (mainly singlet oxygen), causing destruction of cellular targets through direct cellular damage, vascular shutdown and activation of an immune response against targeted cells. Current clinical applications of PDT include the treatment of numerous cancerous and non-cancerous diseases such as age-related macular degeneration or endometriosis. For over 30 years, the use of PDT for treatment of bacterial and fungal infections has been in practice.[1–4]

Although some photosensitizers used in PDT reveal certain tumor selectivity, it is noteworthy that their preferential accumulation in tumors is itself not a guarantee of selective photoinduced tumor damage and successful PDT. The photosensitizers accumulate also in healthy tissues, resulting in uncomfortable adverse effects, such as phototoxic and photoallergic reactions. To avoid this obstacle, a new approach for drug delivery in PDT, called targeted photodynamic therapy (TPDT), has been developed. The aim of TPDT is a specific action towards well-defined targets or biologic pathways that, when inactivated, cause regression or inhibition of the disease process.[1,2]

The commonly applied strategy to increase the specific accumulation of photosensitizers at the target site is encapsulation or attachment of photosensitizers to molecules or molecular constructs which improve affinity of these dyes to the target tissues.[1] This strategy, called targeting, is usually divided into "passive" and "active" ones (Fig. 1). Passive targeting is promoting of drug entry into the tumor cells determined by physicochemical factors of drug carrier, such as material composition, size and surface properties (*e.g.* electric charge) and by pathophysiological factors of the organism, such as tumor microenvironment as well as enhanced permeability and retention (EPR) effect, whereas active targeting involves drug delivery to the specific target sites based on molecular recognition.[1,3] The third strategy of targeting photosensitizers to the tumor cells is the use of photosensitizers alone or attached to carrier systems, which create active forms and produces cytotoxic effects only at the site of the lesion. Some authors suggested terming such delivery systems as "active" in

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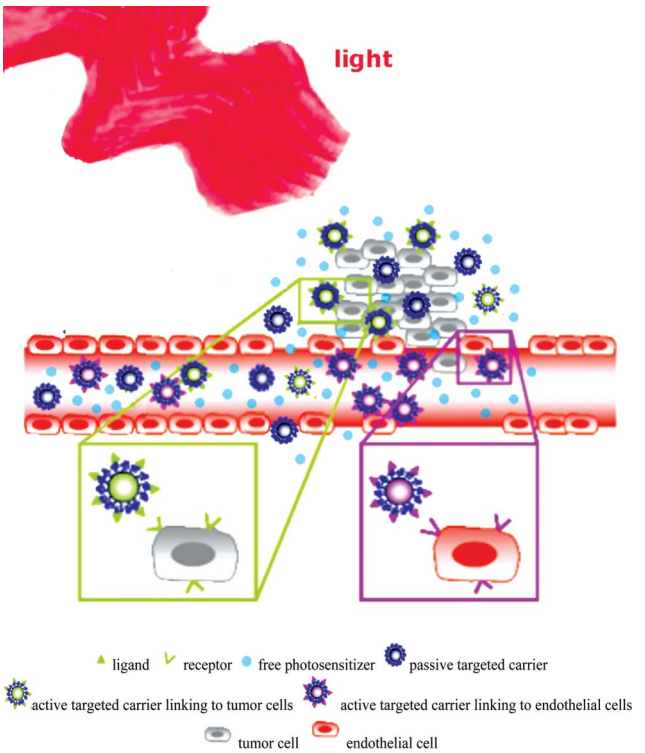

Fig. 1 Schematic presentation of passive and active TPDT. Photosensitizers alone or attached to non-liganded carriers can reach tumors selectively through the "leaky" vasculature surrounding the tumors (EPR effect – passive targeting). The ligands grafted at the surface of carriers allow active targeting by binding to receptors overexpressed by tumor cells or angiogenic endothelial cells (according to ref. 13 and 14).

contrast to "passive" ones, whose role is solely selective transport of photosensitizers to the target sites.[3] However, it seems more precise to call these systems "activatable", because over the site of lesion they are photodynamically inactive. Moreover, it avoids confusion with the traditional differentiation between "passive" and "active" targeting systems, which is commonly used in pharmaceutical sciences.[4]

2. Biodegradable nanocarriers in passive TPDT

Passive targeting makes use of the morphological and physiological differences between normal and tumor tissue to deliver the drug to a target site or utilize localized delivery (Fig. 1).[5] The tumor vasculature is very different from the normal vasculature. Unlike the tight endothelial lining in normal tissues, blood vessels in tumors have gaps as large as 600–800 nm between adjacent endothelial cells.[5] The tumor vessels are often dilated and convoluted, they may have fenestrations and discontinuous membranes. This defective vascular architecture coupled with poor lymphatic drainage induces an enhanced permeability and retention effect (EPR), by which photosensitizers attached to macromolecules can selectively accumulate the tumor interstitium. Another contributor to passive targeting is the unique microenvironment surrounding tumor cells. Fast-growing, hyperproliferative cancer cells show a high metabolic rate, and the supply of oxygen and nutrients is usually not sufficient for the cancer cells to maintain growth. Therefore, tumor cells use glycolysis to obtain extra energy, resulting in an acidic environment. Finally, cancer cells express and release unique enzymes such as matrix metalloproteinases, which are implicated in their movement and survival mechanism.[5–7]

Dramatic increase in tumor drug accumulation – usually tenfold or greater – can be achieved when a drug is delivered by a nanoparticle. Interest in nanoparticles as drug carriers has been increased in recent years. According to common resolution of the International Organisation of Standardisation and of the European Standardisation Committee, nanoparticles may be defined as objects with all three external dimensions in the size range from approximately 1 to 100 nm.[8] In nanomedicine size dimensions of 1–1000 nm are included; this is due to the fact that in medicine nanotechnology aims to improve and optimize material properties for their interaction with cells and tissue to allow, for example, passive tumor targeting or to improve the bioavailability. This approach makes use of nanoscale materials larger than 100 nm.[9] Nanoparticles in a mean diameter of 100 nm show prolonged blood circulation and poor extravasation and are not cleared by reticuloendothelial and phagocytic systems. Hence, drugs encapsulated in nanoparticles can easily accumulate in the organism and its pharmacokinetic parameters such as elimination half-life and volume of distribution often have significantly higher values when compared to the free drug.[10,11] More importantly, nanoparticles can selective accumulate in tumor cells due to the EPR effect. A "leaky", highly fenestrated endothelial wall of tumor vasculature allows selective uptake of nanoparticles by tumor tissue, in contrast to vasculature of healthy tissues being a primary delivery barrier for nanoparticles due to limited pore size. Even nanoparticles greater in size than 100 nm, which are easily cleared by the reticuloendothelial system (RES) and will not leave normal blood vessels, will tend to accumulate in tumors which have relatively "leaky" vascular beds.[10–12] Moreover, by contrast with many free photosensitizers which – due to their small size – can freely diffuse from the tumor cells to the blood vessels, so that concentration of these drugs in tumor tissue may rapidly decrease below the effective concentration, the nanocarriers cannot easily diffuse back into the blood stream because of their large size, resulting in their progressive accumulation in the tumor tissue [13,14] (Fig. 1). For these reasons, nanoparticles are largely applied in TPDT. Traditionally, these constructs can be classified by material composition into biodegradable and non-biodegradable.[3,15]

2.1. Biodegradable nanoparticles

Biodegradable nanoparticles have received a lot of attention due to their possibility of controlling the drug release, versatility in material manufacturing processes and high drug loading.[15,16] They are made of organic natural or synthetic substances that are degraded in the biological environment due to enzyme-catalyzed hydrolysis and hence release the photosensitizers. The chemical and physical structure of these materials can be tailored to accommodate photosensitizers with varying degrees of hydrophobicity, molecular weight, charge and pH.[17]

Liposomes, which are the most intensively investigated family of drug carriers, are uni- or multilamellar lipid vesicles in size of 50–150 nm allowing incorporation of both hydrophilic and hydrophobic substances to improve their drugabililty. Several studies demonstrated a high and fast accumulation of liposomes in tumor

tissues. Lasalle *et al.* studied pharmacologic effects of Foslip, a formulation of 5,10,15,20-tetrakis(*meso*-hydroxyphenyl)chlorin (m-THPC) incorporated in liposomes based on the mixture of dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylglycerol (DPPC/DPPG), on EMT6 tumor bearing mice.[18] After intravenous administration of m-THPC in this formulation at a dose 0.3 mg kg^{-1} the volume of distribution was about 4-fold higher compared to those observed by Jones *et al.*[19] for standard preparation Foscan® (709 and 172 ml kg^{-1}, respectively), suggesting that some tissues preferentially accumulate Foslip.[18] The tumor m-THPC concentration reached its maximal values at 72 h after administration while in the case of Foscan® maximal tumor concentrations were attained 24–48 h after administration.[19,20] However, the best tumor response was estimated for a drug-light interval of 6 h, for which photosensitizer was present both in vasculature and tumor cells[18] similar to the results obtained for Foscan®[19] suggesting that the presence of m-THPC in both endothelial and parenchyma cells is required for optimal PDT efficiency. The release of m-THPC from Foslip liposomes was slower than that from Visudyne® liposomes which are made of more fluid lipids: dimyristoylphosphatidylcholine/egg phosphatidylglycerol.[21]

Conventional liposomes, mainly composed of phospholipids and cholesterol, often exhibit a plasma half-life too short for efficient uptake by tumor cells, because of rapid clearing by RES, and because of disintegration due to lipid exchange with blood plasma lipoproteins and due to molecular interaction between liposome components.[22] Thus, during the past decade, the interest of polymer-based drug delivery systems has grown dramatically with the advent of biodegradable polymers, which are degraded in the biological environment and hence release the photosensitizers. The resulting constructs may have different structures, including micelles and dendrimers.[3,15]

Block copolymer micelles emerge as more attractive drug delivery systems than liposomes, due to their higher stability and small uniform particle size which accomplishes their passive targeting due to the EPR effect and prevents their recognition by macrophages and protein, prolonging their circulation time in blood.[23,24] They are typically spherical nanosized (diameter 10–100 nm) supramolecular assemblies of amphiphilic copolymers, in which the drug may be either confined to a cavity surrounded by a polymer membrane (nanocapsules) or uniformly dispersed in a matrix (nanospheres). The core of these micelles is a loading space that accommodates hydrophobic drugs and the hydrophilic outer shell facilitates dispersal of the micelles in water.[25]

Among synthetic polymers for preparing these micelles, poly(ethylene glycol) (PEG), polylactide (PLA), and their copolymer poly(D,L-lactide-*co*-glycolide) (PLGA) have been studied especially due to their versatility, mechanical strength, biocompatibility, bioresorbability, high drug loading and possibility of controlling the drug release. Cohen *et al.* encapsulated 5,10,15,20-tetrakis(*meso*-hydroxyphenyl)porphyrin (m-THPP) into PEG-PLA copolymer to obtain micelle nanoparticles (a size of about 30.6 ± 3.3 nm) which exhibited phototoxic effect towards head and neck cancer cells.[26] The nanoparticles loaded with 5 and 10% of m-THPP revealed <30% dark toxicity and >90% phototoxicity at a micelle concentration 2–20 mg L^{-1} compared to non-treated cells. Effect of free m-THPP was not studied. No significant cytotoxic effect both for light ($\lambda = 420$ nm) and for nanoparticles without photosensitizer alone were observed.[24] Master *et al.* encapsulated hydrophobic silicon phthalocyanine (Pc4) in PEG-PCL micelles (a mean diameter 73–103 nm) revealing at concentration 400 nM upon irradiation with red light, a significant phototoxic effect ($p < 0.01$) towards MCF-7c3 human breast cancer cells as compared with analogous effect of standard Pc4 formulation in dimethyl formamide (DMF).[27] In contrast to delivery in DMF solution, after which photosensitizer was preferentially localized in mitochondria, endoplasmic reticulum and Golgi apparatus, Pc4 delivered to cell cultures in PEG-PCL nanoparticles was partially distributed to lysosomes. It demonstrates the promise of this carrier for tumor-targeted delivery of Pc4 for site-selective PDT.[27]

Although synthetic polymers might be preferable to use as drug delivery systems due to the possibility of adjusting their mechanical properties and degradation kinetics appropriately for various applications, natural polymers such as agar, albumin, alginate, chitin, chitosan, collagen, cyclodextrins, dextran and gelatin remain attractive because they are relatively inexpensive, readily available and capable of a multitude of chemical modifications.[17,28,29] The use of natural biodegradable polymers to deliver photosensitizers will continue to be an area of active research despite the advance in synthetic biodegradable polymers.[17] Chitosan, the product of partial deacetylation of the natural polysaccharide chitin, presents enhanced tumor target specificity and high ability for encapsulating hydrophobic photosensitizer into the multicore of nanoscale particles.[30] Lee *et al.* prepared the PpIX encapsulating chitosan-based nanoparticles with average size of 290 nm, which were rapidly taken up by SCC7 (squamous cell carcinoma) cells and did not reveal dark cytotoxicity towards these cells while following irradiation with visible light they were highly phototoxic. In SCC-tumor bearing mice PpIX-chitosan-based nanoparticles exhibited enhanced tumor specificity and increased therapeutic efficacy compared to free PpIX.[31] Recently, Hu *et al.* demonstrated significantly higher uptake of chlorin e6 encapsulated into stearic acid-grafted chitosan micelles by A-549 lung cancer cells *in vitro* when compared with uptake of free photosensitizer. The average micelle size (302–330 nm) decreased by 10% with increase of drug content from 5 to 20%.[32] Similarly, alginates, the polysaccharides isolated from brown algae, might be useful for the sustained and localized delivery of photosensitizers.[33] Khdair *et al.* showed that encapsulation of methylene blue in alginate nanoparticles containing anionic surfactant Aerosol®OT (dioctyl sodium sulfosuccinate; an average diameter of 79 nm) enhanced its anticancer photodynamic efficiency *in vitro*.[34]

The new class of biodegradable non-polymeric nanoparticles consists of solid lipid nanoparticles (SLN), which are particles of solid lipid matrix with an average diameter in the nanometre range (150–170 nm). Their excellent physical stability, protection of incorporated labile drugs from degradation, controlled drug release (fast or sustained), good tolerability and site-specific targeting make SLN good candidates for TPDT.[35,36] Despite this, the results of studies performed to date are less promising. As shown by Küchler *et al.*, incorporation of Nile blue in SLN decreased its penetration through pig skin 4–6-fold compared to dendritic core multishell formulation. It was probably due to interactions between lipids of SLN and skin.[36]

Dendrimers are another family of particulate carriers which have aroused increasing interest.[25] They are regularly branched

three-dimensional tree-like structures composed of a central core molecule with a number of functional groups attached to repeated polymer branches organized in concentric layers called "generations" and terminated with surface functional groups which to a considerable degree determine the dendrimer's physicochemical properties.[37,38] Dendrimers can host a variety of carrier molecules, both hydrophobic and hydrophilic. A reasonable cost of manufacturing, good toxicological profile and biocompatibility as well as their controlled multivalency provide attachment of a variety of targeting compounds in a well-defined manner, distinguish them from other nano-sized species used for TPDT. Such formulations may significantly improve the circulation time of photosensitizers and their accumulation in hyperpermeable lesions due to the EPR effect.[39] Battah *et al.* prepared well-defined dendritic molecules for delivery of aminolevulinic acid (ALA), natural precursor of photosensitizing porphyrin PpIX. This delivery vehicle carrying 18 molecules of ALA resulted in increased production of PpIX and in higher phototoxicity towards tumorigenic PAM 212 keratinocytes and A431 human epidermoides when compared with free ALA.[40] In a murine tumor model the dendrimers induced sustained porphyrin production for over 24 h while the porphyrins induced by free ALA revealed concentration maxima between 3 and 4 h.[41] The obtained ALA dendrimers (molecular weight: 3679) are too low to elicit the EPR effect which can improve tumor selectivity for larger dendrimers and are cleared relatively rapidly from mouse circulation. The rate of release of ALA residues by dendrimer enzymatic hydrolysis (25% at 3 h and 40% at 24 h after intraperitoneal administration to mice in dose 200 mg kg^{-1}) within the cells may be the rate-limiting step for porphyrin production.[41]

It is also possible to encapsulate photosensitizers into dendrimers. The slow degradation of such complexes can give rise to prolonged release *in vivo*. Kojima *et al.* developed two PEG-attached dendrimers derived from poly(amidoamine) (PAMAM) and polypropylene imine (PPI) dendrimers (average molecular weight: 2000) to encapsulate rose bengal and PpIX. The PEG-PPI dendrimers held both these molecules in a more stable manner than PEG-PAMAM ones, probably due to higher hydrophobicity. The dendrimers of PpIX encapsulated in PEG-PPI revealed more efficient phototoxic effect towards HeLa cells when compared to free PpIX.[42]

In contrast to free PpIX, which reaches cell interior by membrane diffusion, the dendrimeric PpIX with cationic and anionic groups entered cells by endocytosis and remained localized in lysosomes.[43] The cationic dendrimers entered Lewis lung carcinoma (LLC) cells 22–25 times more rapidly and revealed higher phototoxicity 230 times towards these cells than anionic ones. The observed differences of cell uptake and phototoxicity of both dendrimeric compounds was probably due to different electrostatic association of cell membranes. The cationic dendrimer could strongly adsorb on negatively charged membrane components (*e.g.* glycoproteins) through electrostatic interactions, while the anionic ones could reveal lower affinity to these membrane components, due to electrostatic repulsion. As a consequence, positively charged dendrimers may be more strongly bound by plasma membranes and may cause photodamage of these membranes more vigorously than anionic ones, although quantum yields of $^1O_2$ production were similar for both positively and negatively charged PpIX dendrimers and for free PpIX (0.49, 0.41 and 0.45, respectively).[43] Compared to free PpIX, its cationic dendrimer revealed a phototoxic effect 20 times higher towards LLC cell plasma membranes, probably connected with lipid peroxidation, protein cross-linking, loss of ionic homeostasis and release of hydrolases to cytoplasm from damaged lysosomes. Thus cationic dendrimer porphyrins seem to be a new class of promising PDT photosensitizers.[43]

To improve the passive targeting of this promising PDT system, cationic dendrimer porphyrins have been assembled with linear copolymers of poly(ethylene glycol)-poly(a,b-aspartic acid) to obtain micellar constructs with a diameter of *ca.* 55 nm which prolongs circulation time of carriers in the blood and increases their tumor accumulation by EPR. The PDT efficiency of such dendrimer porphyrins was 40-fold higher than free dendrimers, probably due to decreased tendency of these micellar dendrimers to aggregate, resulting in a higher yield of singlet oxygen production.[44] Nishiyama *et al.* synthesized dendrimeric phthalocyanines with diameter of *ca.* 50 nm which accumulated in the endosomes and efficiently induced phototoxic effects towards human adenocarcinoma cells *in vitro* and towards A549 tumors in mice without inducing skin phototoxicity. The development of dendrimers as carriers with smart functions may be a key to further advance the clinical application of PDT.[45]

2.2. Non-biodegradable nanoparticles

Compared to biodegradable polymeric carrier system, non-biodegradable nanoparticles have several advantages, due to their facile synthesis, ease of functionalization, and biocompatibility.[3,15] These nanoparticles act as catalysts to produce free radicals from dissolved oxygen. They are not destroyed by the treatment process, thus they may be used repeatedly with adequate activation. Their particle size, shape, porosity and monodispersibility can be easily controlled during preparation, and exquisite control over pore size allows oxygen diffusion in and out of the particle but is not good for the drug to escape. Moreover, they are not susceptible to microbial attack.[3,15] Most non-biodegradable nanoparticles are ceramic- or metallic-based.

Silica-based nanoparticles have successfully encapsulated photosensitizers such as m-THPC,[46] Fotolon®[47] and PpIX.[48] Roy *et al.* demonstrated significantly higher uptake of 2-devinyl-2-(1-hexyloxyethyl)pyropheophorbide (HPPH) incorporated in ultrafine organically modified silica-based nanoparticles (diameter ~30 nm) by HeLa cells when compared with free photosensitizer.[49] The methylene blue-encapsulating silica nanoparticles with a mean diameter of 105 nm were able to induce photodamage of HeLa cells under irradiation with light of 635 nm and revealed near-infrared fluorescence within the xenograft tumor in mice.[50]

Gold nanoparticles are promising nanocarriers for therapeutics. Cheng *et al.* synthesized conjugates of pegylated gold-nanoparticles with average diameter 5.0 nm, which can act as water-soluble and biocompatible "cages" allowing delivery of hydrophobic photosensitizers to its site of PDT action. Pc4 conjugated with these nanoparticles reached the skin tumors in a murine model through a passive process and the time of maximum drug accumulation has been reduced to only <2 h, compared to 2 days for the free photosensitizer. When the pegylated nanoparticles circulate in the body they can escape uptake by the RES. This suggests that the pegylation of polymer-photosensitizer constructs may improve their tumor targeting.[51]

Wieder *et al.* reported the development of delivery systems based on gold nanoparticles whereby the phthalocyanine photosensitizer was bound to the surface of the nanoparticle. These conjugates were easily taken up into HeLa cells, and upon irradiation, a decrease in cell viability by 57% was observed compared to the non-irradiated cells, while free phthalocyanine decreased viability of irradiated HeLa cells only by 26% in comparison to intact cells.[52] This significant improvement in PDT efficiency is probably due to the 50% enhancement of singlet oxygen quantum yield observed for the phthalocyanine-nanoparticle conjugates as compared to the free photosensitizer. These results suggest that gold nanoparticle conjugates hold great potential as a delivery vehicle for photosensitizers in PDT.[52]

Among non-biodegradable organic polymers applied for drug delivery, the best characterized are co-polymers of *N*-2-hydroxypropyl methacrylamide (HPMA). These co-polymers used as drug carriers possess a size <10 nm and circulate in the blood system for prolonged period of time and by means of EPR they localize to tumors effectively and selectively.[53,54] Conjugates of drugs with HPMA co-polymers have *in vivo* high antitumor activity. Detailed studies were performed of pharmacokinetics and photodynamic activity of HPMA conjugates with *meso*-chlorin e6 monoethylene diamine (m-Ce6). These studies demonstrated that HPMA substantially improves biodistribution of m-Ce6 and in the process improves the therapeutic index of PDT against ovarian carcinoma cells.[55]

The major limitation of passive targeting is poor transfection efficiency at the target site after systemic administration. The lower size particles (<5 nm) are rapidly excreted through the renal filtration system and therefore cannot maintain stable circulation in the bloodstream. Furthermore, the EPR effect is strongly influenced by heterogeneity of tumor morphology and physiology, because permeability of vasculature varies both within and among tumors. For instance, in very young tumors which have not yet developed a vascular system, the use of passive targeting was ineffective.[56] Thus, to overcome these shortages and to improve uptake of photosensitizers by treated cells, active targeting has been employed.[57]

3. Carrier systems for active TPDT

Active targeting consists in transporting drugs to target cells using specific ligands which bind to appropriate receptors expressed at the target site. Targeting ligands are chosen to bind to receptors overexpressed by tumor cells or tumor vasculature and not expressed by normal cells (Fig. 1). Moreover, targeted receptors should be expressed homogeneously on all targeted cells.[13,58]

Photosensitizers linked to peptides that possess high affinity to cell receptors can enhance accumulation of these dyes in tumor tissues *via* receptor-mediated endocytosis. A range of peptide sequences have been used successfully to direct photosensitizers to target both tumor vessel and tumor cell receptors.[57,59]

3.1. Tumor vessel-targeted PDT with use of peptide carrier system; antiangiogenic factors

There are several advantages of targeting the tumor vasculature as compared to targeting tumor cells (Fig. 1). Targeting the vasculature allows physiological barriers that prohibit dissemination of photosensitizer through the tumor to be overcome and diminishes secondarily acquired drug resistance due to limited susceptibility of neovascular endothelial cells to undergoing phenotypic variations. Furthermore, destroying the vasculature decreases the growth and metastatic capabilities of the tumor. Finally, the tumor vasculature is not specific for the type of cancer.[60]

Hypoxia and other mechanisms, such as genetic mutations, oxidative and mechanical stress or glucose deprivation, induce a variety of growth factors and cytokines able to stimulate angiogenesis.[61] Angiogenesis, characterized by the invasion, migration and proliferation of endothelial cells to degrade the basement membrane and to form a new lumen structure, appears to be one of the crucial steps in tumor translation to the metastatic form, capable of spreading to other parts of the body. Thus, targeting of angiogenesis has become a large area of focus for cancer therapeutics.[53] The main angiogenic targets explored in TPDT are vascular endothelial growth factor receptor (VEGFR) and $\alpha_v\beta_3$-integrin.

Receptors for growth factors are often overexpressed on cancer cells, representing an excellent target for specific photosensitizer delivery systems. Vascular endothelial growth factor (VEGF) is considered to be the key mediator of angiogenesis in cancer. Thus, after conjugation with photosensitizer it could potentiate the vascular effect of PDT that is thought to play a major part in tumor eradication. The conjugation of 5-(4-carboxyphenyl)-10,15,20-triphenyl chlorin (TPC) to a ATWLPPR heptapeptide, specific for the VEGF co-receptor neurophilin-1, significantly enhanced cell uptake and photodynamic activity when compared to free TPC. In nude mice xenografted with U87 human malignant glioma cells expressing VEGF receptors, the conjugated photosensitizer could target not only angiogenic endothelial cells but also tumor cells.[62]

The $\alpha_v\beta_3$-integrin, a heterodimeric transmembrane glycoprotein receptor, is over-expressed in many tumor cells, such as osteocarcinoma, neuroblastoma and lung carcinoma. Chaleix *et al.* synthesized four porphyrin derivatives bearing the $\alpha_v\beta_3$-integrin ligand RGD tripeptide. Three of these porphyrin derivatives revealed photodynamic activity on K562 leukemia cells to a degree comparable to that of Photofrin®. The same authors described the synthesis of a cyclic peptide containing the RGD sequence and showing an increased affinity for integrins. Carboxy-glucosyl porphyrins coupled to this peptide showed the same efficiency for $^1O_2$ production as hematoporphyrin.[63]

Hu *et al.* developed a TPDT system by conjugating factor VII protein with verteporfin (VP).[64] Factor VII (fVII) is a natural ligand for the receptor tissue factor (TF) with high affinity and specificity. The reason for targeting TF for the development of TPDT is that TF is a common but specific target on angiogenic tumor vascular endothelial cells (VEC) and many types of tumor cells, including solid tumors and leukemia. PDT with use of fVII-VP conjugates could selectively kill TF-expressing breast cancer cells and VEGF-stimulated angiogenic human umbilical vein endothelial cells (HUVEC) but had no side-effects on non-TF expressing non-stimulated cells. The PDT effect toward mouse breast cancer cells was 3–4-fold greater when compared with the effect of free photosensitizer. Since TF is expressed in many types of cancer cells including leukemic cells and, selectively, on angiogenic tumor VEC, TPDT using fVII conjugates could have broad therapeutic applications for cancer treatment.[64]

3.2. Tumor cell-targeted PDT with use of peptide carrier system

Cell proliferation markers are significant targets for cancer therapeutics, as many of these markers are highly overexpressed in certain tumor cells. Targeting highly expressed ligands and their receptors is a promising area that can ensure the elimination of highly malignant tumors and metastatic cells that have not become large enough to induce angiogenesis.[57]

Upon systemic administration, many photosensitizers can be readily incorporated into lipoproteins such as low-density lipoproteins (LDL) with the receptors being more abundant in tumor tissues than in the surrounding normal cells. Loaded with photosensitizers LDL can be targeted to both neovascular endothelial cells and tumor cells displaying a high expression of LDL receptors due to increased cell proliferation. The role of LDL receptors as carrier molecules to improve phototoxicity has been investigated using various photosensitizers, including hematoporphyrin derivative, zinc phthalocyanine, benzoporphyrin derivative and chlorin e6 (Ce6).[16] Zheng *et al.* successfully reconstituted the conjugates of pyropheophorbide *a* with cholesterol oleate and demonstrated that this photosensitizer reconstituted LDL can be internalized *via* LDL by human hepatoblastoma G_2 tumor cells.[65]

The use of lipoproteins as carriers for photosensitizer delivery to target tumor tissues imposes certain limitations, connected with redistribution in the blood, depending on dynamics of interactions between photosensitizers and blood components, which are not yet fully understood.[59,66] Furthermore, such a mode of delivery predetermines to a large degree the subsequent subcellular distribution and thereby its sites of action. An increasingly popular alternative approach is conjugating photosensitizers to monoclonal antibodies.[16,59]

Antibody-targeted PDT is an established technique that improves photosensitizer delivery through photosensitizer conjugation to targeting antibodies. The antibodies then deliver the photosensitizers to specific antigens over-expressed on target cells. Despite promising results and years of progress, antibody-targeted PDT has yet to see clinical implementation.

Hydrophilic photosensitizers are most suitable for photoimmunotherapy because of their solubility in water. Vrouenraets *et al.* revealed phototoxicity of hydrophilic derivative of *meso*-5,10,15,20-tetrakis(*N*-methyl-4-pyridyl)porphine ($TMPyP_4$) conjugated with monoclonal antibody 425, recognizing epidermal growth factor receptors towards vulvar cells, contrary to free photosensitizer which was not efficiently taken up by these cells.[67] Bhatti *et al.* synthesized conjugates of pyropheophorbide a with single-chain Fv antibody fragments, which led to significant regression of breast cancer tumors upon irradiation.[68]

If photosensitizer-antibody conjugates have been the subject of the most intense investigations in the past, they appeared to present some major limitations: large size and thus poor tumor penetration, nonspecific uptake of the antibody molecules by liver and reticulo-endothelial system and, often, the absence of cellular internalization. One challenge is that the antibodies must have a low photosensitizer-to-antibody conjugation ratio to maintain targeting function.[16,59] Thus, research has focused on the targeting of receptors – rather than antigens – that are preferentially expressed in tumor tissues. In spite of numerous encouraging *in vitro* results with use of proteins as ligands, peptides are molecules that have widely been described for targeted therapy and that now appear to be interesting candidates for TPDT.[16]

The most established cell proliferation targets used for actively targeting photosensitizers include human epidermal growth factor receptor (EGFR) and transferrin receptors.[59]

Receptors for growth factors are often overexpressed in cancer cells, representing excellent targets for specific photosensitizer delivery systems. Epidermal growth factor receptor (EGFR) is widely expressed in many human tumors, particularly in glioblastoma multiforme and in many epithelial tumors, such as head and neck, breast, renal cell or esophageal cancers.[69,70] This makes EGFR an important target for treatment of the type of cancers given above and epidermal growth factor (EGF) – a potent mitogenic and angiogenesis-stimulating factor – a potential drug carrier. The conjugate of disulfochloride aluminium phthalocyanine with mouse EGF were seven times more phototoxic against human breast carcinoma cell line MCF-7 than free disulfochloride aluminium phthalocyanine.[71] As human EGF, in contrast to that of mice, may lose its biological activity due to presence of two amino groups in the lysyl residue after direct conjugation to photosensitizer, Gijsens *et al.* conjugated tin(IV) chlorin e6 monoetylene diamine (SnCe6(ED)) with EGF through human serum albumin (HSA) as a linker.[72] This conjugate showed a potent phototoxicity (IC_{50} = 63 nM) towards MDA-MB-468 human breast adenocarcinoma cells dependent on EGF, because free SnCe6(ED) and SnCe6(ED) conjugated only to HSA revealed no phototoxic effect against these cells.[72]

Transferrin is a blood plasma glycoprotein for delivery of ionic iron. It is especially useful in targeting to cancer cells, since many cancer cells overexpress receptors for this protein on their surface. Bioconjugates composed of transferrin and hematoporphyrin were found to induce phototoxicity in erythroleukemic cells and the surviving cells did not reveal resistance to subsequent treatment with these conjugates.[59] The aluminium phthalocyanine tetrasulfonate encapsulated in distearoyl phosphatidylethanolamine-PEG liposomes conjugated to transferrin developed 10-fold higher photodynamic effect than free photosensitizer, while the same photosensitizer in non-targeted liposomes revealed no photodynamic activity, whereas analogous transport of hypericin by transferrin-coupled liposomes was impossible due to instability of sensitizer in the liposomal membrane.[73]

Rahimipour *et al.* described the coupling of protoporphyrin IX to peptides acting as gonadotropin-releasing hormone (GnRH) agonists or antagonists with the goal to selectively target GnRH receptors as assessed *in vitro* by the assays with use of radioligands.[74] The GnRH receptors are largely overexpressed in prostate and breast tumors.[74] The affinity of photosensitizer-peptide conjugates was found to be lower than that of the corresponding peptides, however their photodynamic activity was increased about 1.5-fold in GnRH-expressed pituitary gonadotrope cells when compared with unconjugated PpIX. Interestingly, in addition to their tumor-targeting properties the peptide-photosensitizer conjugates acted on the luteineizing hormone levels.[74]

According to Oleinick and Evans, the photosensitizers of greatest interest in PDT bind to various cytoplasmic membranes but are not found in the cell nucleus and do not bind to DNA.[75] Although it is believed that the key targets of singlet oxygen oxidative damage in PDT are mitochondria,[76,77] some authors

claim the cell nucleus is a more sensitive site for $^1O_2$ damage than other cell organelles.[78] On the other hand, some photosensitizers may concentrate near the cell nucleus[79] and bind to nucleic acids.[80,81] Apart from this, PDT may induce DNA damage and cell mutagenicity, the extent of which is dependent on the properties of photosensitizer,[82–86] of cellular repair mechanisms[85,86] and of target gene.[87]

Proximity of photosensitizer to the nucleus by around 20 nm corresponding to average intracellular diffusion distance of $^1O_2$ producing during PDT effect[88] results in enhanced toxicity of tumor cells.[89] Hence, creation of $^1O_2$ in close proximity to the cancer cell DNA would dramatically increase the odds of tumor cells. Therefore, efforts have been made to increase photosensitizer delivery to the cell nucleus. One of the results of these efforts are nuclear localization signal peptides (NLS-peptides).[90]

3.3. Nuclear localization signal peptides (NLS-peptides)

The proteins to be imported into the nucleus must have a NLS or an analogous amino acid sequence, be resistant to proteolysis, and be able to be translocated to the nucleus in their native conformation without requirement of molecular chaperones or folding enzymes. To date, different interesting NLS peptides have been synthesized, such as loligomers, branched peptides incorporating identical proteins arms coding for functional domains which can guide nuclear uptake of photosensitizers.[91] Incorporation of Ce6 into loligomers enhanced its phototoxicity 400-fold towards Chinese hamster ovarian cells when compared with effect of free photosensitizer, although it was unclear if this enhancement was a consequence of nuclear localization.[92] Sobolev's group designed a series of Ce6 conjugates with NLS endosomolytic peptides enabling to circumvent lysosomal trafficking. The most efficient photosensitizing agent was Ce6-insulin adduct, containing the coding sequence of NLS. This conjugate efficiently targeted the cell nucleus and its phototoxicity was 2400-fold higher than that of free Ce6.[93]

3.4. Aptamers

Similar to peptide-directed targeting, aptamer-based nucleic acid targeting seems to be a promising and powerful targeting technique. Aptamers are short nucleotides that fold into well-defined three-dimensional architectures, enabling specific binding to extra- and intracellular targets as well as to membrane constituents and receptors.[94] In contrast to antisense oligonucleotides and small interfering RNAs (siRNAs), aptamers bind to existing protein and non-protein (*e.g.* aminoglycosides or theophyllin[95]) targets with high affinity and specificity, analogous to monoclonal antibodies. They have the advantage of smaller size, ease of isolation and lack of immunogenicity. Moreover, aptamers are structurally stable across a wide range of storage conditions, maintaining the ability to form their unique tertiary structures.[96,97]

Shieh *et al.* prepared conjugates of G-quadruplex AS1411 aptamers with $TMPyP_4$.[97] These conjugates revealed substantially higher affinity to MCF7 breast cancer cells when compared with normal epithelial cells. After irradiation with blue light the photodamage to MCF7 cells was larger than to M10 epithelial cells. These results indicated that use of aptamer-photosensitizer complexes interacting uniquely with nucleolin on the cell surface may be a potential tactic in cancer therapy.[97]

Ferreira *et al.* designed aptamers that are only internalized by epithelial cancer cells and can be precisely activated by light to kill such cells.[98] Phototoxic DNA aptamers were selected to bind to unique short O-glycan-peptide signatures on the surface of breast, colon, lung, ovarian and pancreatic cancer cells. These surface antigens are not present on normal epithelial cells but are internalized by cancer cells thus providing a focused mechanism for their intracellular delivery. When modified with Ce6, these aptamers exhibited a >500-fold increase in phototoxicity compared to the free photosensitizer and were non-cytotoxic towards cells lacking O-glycan-peptide markers.[98] Thus, aptamers can serve as delivery vehicles in precisely routing cytotoxic carriers into epithelial cancer cells, from which arise the majority of cancers.

Despite their advantages, fewer functional aptamers have been identified compared with antibodies. Thus, many oligonucleotide aptamers that are important for cancer research are not yet available. The susceptibility of aptamers to nuclease degradation is their major pitfall. Although the incorporation of chemically modified nucleotides at specific points along the nucleotide chain increases resistance to nucleases, this also makes the chemical synthesis of functional aptamers difficult and costly.[2] As an attractive alternative, many research groups highlighted the utility of vitamins as targeting ligands for sensitizer delivery.

3.5. Folic acid

Among vitamins, folic acid seems to hold better promise in TPDT. It is stable in storage and circulation, inexpensive, non-toxic and non-immunogenic. Folic acid has a high affinity for folate receptors which are up-regulated in numerous cancers, such as ovary, kidney, lung, breast and brain carcinomas, and at the same time are absent in most normal tissues. Moreover, folic acid can be easily conjugated with PDT sensitizers. Schneider *et al.* synthesized conjugates of monocarboxylic acid tetraphenylporphyrin with folic acid, which were taken up by KB nasopharyngeal cells 7-fold as much as free photosensitizer. These conjugates showed also significant photodynamic effects against KB cells while free tetraphenylporphyrin showed no photodynamic action at the same conditions.[99]

Stevens *et al.* synthesized folate receptor-targeted SLN (a mean diameter <200 nm) as a carrier for lipophilic derivative of hematoporphyrin in folate receptor overexpressing tumor cells. The results of *in vitro* study showed that introduction of folic acid into hematoporphyrin-stearylamine SLN greatly increases phototoxicity and cellular uptake in FR-positive KB cells when compared with non-functionalized nanoparticles. Additional pharmacokinetic and photodynamic effect studies are necessary.[100]

As was mentioned above, precise control of intracellular site of $^1O_2$ production may be essential for cytotoxic effect of PDT. To create the possibility of such control, a new class of photosensitizers has been developed, called activatable photosensitizers.

4. Activatable photosensitizers

Activatable photosensitizers may be turned on by a wide variety of molecular stimuli, resulting in increased cytotoxic singlet

oxygen generation. They can more potently and specifically kill diseased cells that differ from normal cells with respect to their environment, enzyme expression, or nucleic acid expression.[4,101,102]

4.1. Environmental activatable photosensitizers

The concept of stimuli-sensitive delivery systems is based on the fact that tumors usually have a lower extracellular pH than healthy tissues. Delivery systems attached with pH sensitive or thermosensitive components would have the ability to response to local physiological stimuli such as pathology-associated changes in local pH and/or temperature. Shieh *et al.* encapsulated m-THPC in pH-sensitive micelles based poly(2-ethyl-2-oxazoline)-*b*-poly(D,L-lactide) diblock copolymer. In comparison with the release at pH 5.0, the photosensitizer release from micelles at pH 7.4 was effectively suppressed. *In vivo*, the PDT effect was similar to that exhibited by free m-THPC, but encapsulated photosensitizer had less skin phototoxicity.[103] Rijcken *et al.* incorporated solketal-substituted phthalocyanine into thermosensitive micelles made of PEG-HPMA. However, the obtained formulation was hardly taken up by cells.[104]

Environmental activation is an important factor in controlling the singlet oxygen generation of photosensitizers. McDonnell *et al.* synthesized a series of pH-activatable photosensitizers based on electron transfer. These photosensitizers were demonstrated to effectively kill cells.[105] This approach was extended to develop photoinduced electron transfer quenchers that are only active in a hydrophobic environment. The result of this study was a construct consisting of a photosensitizer, a modulatable photoinduced electron transfer quencher, and a protein-targeting ligand that directed this activatable photosensitizer to the inositol triphosphate receptors in cells. The photoinduced electron transfer quencher became inactive upon binding in the hydrophobic pockets of cellular proteins. This approach allows inactivation of specific proteins in living cells.[106]

Another approach to photosensitizer activation was to use two different control points, effectively functioning as a photosensitizer activation logic controller.[107] This activatable photosensitizer was designed to respond to two important physiological parameters – sodium ion concentration and pH – but only when both the hydrogen and sodium ion concentration were high. In this case, iodinated bodipy was attached to crown ether for sodium ion-induced photoinduced electron transfer as well as pyridyl groups for conferring pH sensitivity. This activatable photosensitizer was shown to undergo a >6-fold increase in singlet oxygen at low pH and high sodium ion concentration, but no increase in low pH alone and only partial increase in high concentration of sodium ions alone.[107]

Further research in this field brought development of new classes of photosensitizers, called "photodynamic molecular beacons".[4,101,102,108–110]

4.2. Photodynamic molecular beacons

The concepts of photodynamic molecular beacons (PMB) is an extension of the approach of molecular beacons that use Förster resonance energy transfer (FRET) principle for controlling emission in response to target activation. By combining molecular beacons with PDT, it is possible to enable cancer biomarker-controlled production of singlet oxygen with unprecedented PDT selectivity. The PMB consists of photosensitizer, quencher and disease-specific linker, keeping them in close proximity so that the photosensitizer is quenched due to FRET. When the linker interacts with target molecules, photosensitizer and quencher are separated one from another and the first can be photoactivated.[4,106] Among the linkers described in the literature, the ones which demonstrated the highest efficiency are the openable and cleavable linkers (Fig. 2).

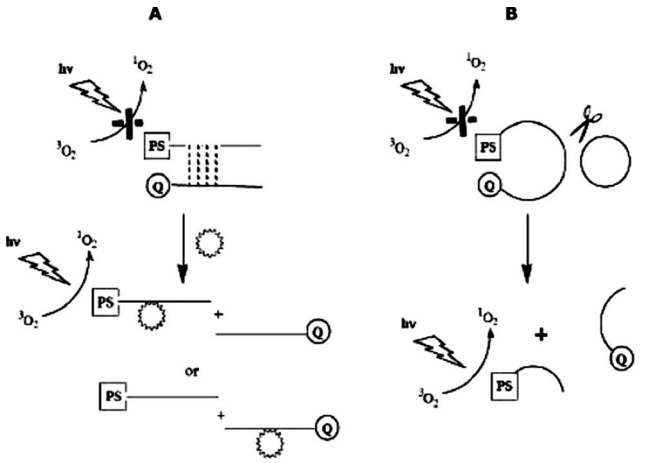

Fig. 2 Activatable photosensitizers with openable and cleavable linker. In the case of openable linker, the moieties of photosensitizer and quencher are separated from each other due to a change of conformation of the linker without cleavage (A), while in the case of a cleavable one both these moieties are released because of cleavage of the linker in the cellular environment (B).[101]

Openable linkers are designed in order to keep the photosensitizer and quencher as close as possible to the biological target; then both moieties are separated using the better affinity of the linker moiety towards the triggered molecule. The openable linkers keep their entire integrity but are bound to the targeted molecules which allow the restoration of $^1O_2$ generation. As openable linkers nucleic acids, due to robust synthesis and well-characterized chemical structure, demonstrate the possibility of reliable and precise control of photosensitizer activation. Since all cancerous and many non-cancerous diseases are connected with gene mutations or altered gene expression, nucleic acid activatable photosensitizers could form the basis of PDT.[4,101,102]

Although some photosensitizers can increase singlet oxygen production simply upon direct binding to nucleic acids,[4] to realize the benefits of acid sequence-specific targeting, a functionalized photosensitizer design is required. Clo *et al.* have developed a PMB based on the DNA reverse hybridization strategy.[111] In this approach, photosensitizer pyropheophorbide-*a* is linked to an oligonucleotide sequence sharing the same sequence as the target. Upon addition of a complementary oligonucleotide conjugated with Black Hole Quencher 3 (BHQ3), one of a new class of designed high-efficiency quenching dyes, the two strands hybridize, forcing the photosensitizer and quencher into close contact and attenuating the $^1O_2$ signal. Upon interaction with the target nucleic acid, the photosensitizer-linked strand is displaced, resulting in photosensitizer unquenching and $^1O_2$.[111] To ensure efficient displacement of the photosensitizer strand, a longer quencher

strand and target strand may be used, facilitating the formation of the activated state of this photosensitizer. For example, a pyropheophorbide-*a* was held in place next to a carotenoid quencher by a 6-base stem with a loop portion specific for the cRaf-1 oncogene. Upon incubation with cRaf-1 expressing cells, PMB entry into the cells was observed and was dependent on the presence of the pyropheophorbide *a* photosensitizer.[110] Zhu *et al.* have designed a new kind of $^1O_2$ production probe by linking non-covalently a ssDNA aptamer-photosensitizer moiety with single-walled carbon nanotubes (SWNT). The aptamer coupled to Ce6 was able to perform $^1O_2$ production upon photosensitizer irradiation. Due to the π-stacking between the aptamer and SWNT, the energy transfer between Ce6 and SWNT led to 98% $^1O_2$ quenching, however production of $^1O_2$ was restored in the presence of thrombin.[112]

Contrary to openable linkers, cleavable ones aim to release the moieties of photosensitizer and quencher (Fig. 2). Enzymes, particularly proteases, are excellent targets for such photosensitizers due to well-characterized catalytic activity. Smaller amino acid peptide sequences that are cleaved by proteases can form the bioactive linker of activatable photosensitizers. The first example of such a photosensitizer geared toward pure PDT purposes used the short peptide approach with a specific amino acid sequence targeting caspase-3, an enzyme involved in apoptosis. The "beacon" consisted of the photosensitizer pyropheophorbide-*a*, a bioactive linker of a specific amino acid sequence, and a quencher – carotenoid or BHQ3. Upon incubation with caspase-3, the peptide portion of the activatable photosensitizers was cleaved and singlet oxygen production increased 4-fold. Because singlet oxygen generation is dependent on irradiation intensity, using a greater light dose may induce apoptosis and caspase activation even with a well-quenched photosensitizer.[108]

The drawback of caspase-targeted PMB is that they do not preferentially target a disease-associated enzyme. Thus, it is necessary to find specific cleavable peptide linkers to target tumor-associated proteases. One result of these finding is that an activatable photosensitizer targeting matrix metalloproteinase 7 (MMP-7), which is associated in many cancers, was developed. Upon incubation of this photosensitizer with MMP-7 the production of singlet oxygen was 19-fold higher, corresponding to the same production induced by quencher-free construct. Inhibition of MMP-7 or lack of its expression in treated cells, as well as modification of linker amino acid sequence, resulted in disappearance of PDT activity.[109] Chen *et al.* have reported the concept of a new PMB based on both folate receptor driving accumulation within cancer cells and the ability of PMB to be activated in the presence of MMPs. This construct should benefit the advantages of the ability of photodynamic beacons to be cytotoxic only within the targeted area.[113]

Activatable photosensitizers have progressed remarkably in a short period of time, but much work is required so they can fulfil their potential.

5. Multifunctional nanoplatforms in TPDT

A big challenge and opportunity for contemporary PDT are multifunctional nanoplatforms in which multiple functionalities of therapeutics, targeting, stimuli responsiveness and imaging can be integrated in one nanoparticle to achieve a more potent target response.[114] Reddy *et al.* have reported the application of a multifunctional nanoplatform for TPDT. In this study, polyacrylamide nanoparticles (with an average particle diameter of 40 nm) for targeting brain tumors were used for encapsulating Photofrin® with iron oxide as the imaging agent. A tumor-homing peptide, F3, which selectively targets tumor cells and angiogenic vasculature, was attached to the nanoparticle surface through a PEG spacer. The F3 targeting moiety significantly enhanced the tumor nanoparticle localization; considerable magnetic resonance imaging contrast enhancement was achieved in intracranial rat 9 L gliomas following intravenous nanoparticle administration. An improved treatment efficiency was observed in the animals, which exhibited a significantly enhanced overall survival in comparison to the animals treated with Photofrin® encapsulated in non-targeted nanoparticles or with free Photofrin®.[115]

An ambitious goal of drug delivery is external "remote control" of the drug carriers in order to achieve the goal of maximum target specificity. One way to achieve this goal is introduction of magnetic properties in drug carrier, following manipulation of pharmacokinetic and pharmacodynamic properties with an external magnetic field. Cinteza *et al.* synthesized multifunctional nanocarrier system, demonstrating combined functions of a magnetophoretically guided drug together with PDT, consisting of polymeric micelles of phosphatidylethanolamine–poly(ethylene glycol) (PE-PEG) loaded with the photosensitizer HPPH and magnetic Fe_3O_4 nanoparticles with an average diameter of 8 nm.[116] The average diameter of empty micelles was about 13 nm and of ones loaded with both HPPH and magnetic nanoparticles was about 35 nm while average diameters of micelles loaded only with photosensitizer or ferric oxide were about 19 and 24 nm, respectively. The *in vitro* study showed that this novel nanoplatform provides a possibility of magnetically guided delivery of photosensitizer to target HeLa cells, and revealed high stability of nanocarrier and high retention of HPPH whose phototoxicity was similar to that of HPPH in Tween-80 micelles and remained unaltered upon magnetic nanoparticle coencapsulation. Thus, incorporating a magnetic moiety in a nanocarrier formulation can offer an additional degree of freedom for targeted drug delivery and consequent therapeutic efficiency.[116] Moreover, such a magnetic component of the nanoplatforms may allow combination of PDT with hyperthermia.[117] Other multifunctional nanoplatforms were also designed, in which PDT was combined with radiotherapy[118] or hyperthermia,[119] as well as PMB for simultaneous treatment and response monitoring (PDT with a built-in apoptosis sensor),[120] however these constructs lack cancer-targeting capabilities.

6. Summary

The increasing cases of cell resistance towards conventional chemotherapeutic drugs and non-specific toxicity of drugs on healthy tissues give impetus to the development of new therapeutic methods. One of these methods is TPDT. Further exploration of this strategy that targets the photosensitizers to diseased cells enhancing the treatment outcomes of PDT may lead to new and improved treatments for a variety of cancers and other diseases.[2,121]

On the other hand, in the face of exceeding progression of contemporary medicine, exploring pathological processes at cellular and molecular levels, it is an imperative to revise old paradigms of PDT. A human solid neoplasia should be regarded

as an intricate yet poorly organized "organoid" whose function is maintained by a dynamic interplay between neoplastic and host cells.[7] Tumors develop their unique anatomical structure and build physiological barriers that reduce the penetration and transport of anti-cancer drugs, especially macromolecular agents. These barriers include poor blood flow in large tumors, tumor capillary wall permeability, elevated interstitial fluid pressure, stroma causing poor diffusion in the interstitium, modified fluidity of cancer cell membrane and heterogeneous antigen expression.[6,7] The availability of oxygen is a critical feature for obtaining the desired photosensitization. Moreover, the presence of hypoxia in tumor tissue may induce a variety of pro-angiogenic cytokines and decrease extracellular pH, influencing in this manner the cytotoxic effect of photosensitizers.[7] Thus, a new paradigm of PDT should be focused on the concepts of heterogeneity and dynamic state of tumor, whose morphology and physiology can intensively change during progression of disease and be strongly influenced by environment factors.[1–4]

Abbreviations

ALA	5-Aminolevulinic acid
BHQ3	Black Hole Quencher 3
Ce6	Chlorin e6
DMF	Dimethyl formamide
DPPC	Dipalmitoyl phosphatidylcholine
DPPG	Dipalmitoyl phosphatidylglycerol
EGFR	Receptor of endothelial growth factor
EPR	Enhanced permeability and retention
FR	Folate receptor
FRET	Förster resonance energy transfer
fVII	Conjugating factor VII
GnRH	Gonadotropin-releasing hormone
HPMA	*N*-2-Hydroxypropyl methacrylamide
HPPH	2-Devinyl-2-(1-hexyloxyethyl)pyropheophorbide
HUVEC	Human umbilical vein endothelial cells
LLC	Lewis lung cancer
m-Ce6	*meso*-Chlorin e6 monoethylene diamine
MMP-7	Matrix metalloproteinase 7
m-THPC	5,10,15,20-Tetrakis(*meso*-hydroxyphenyl)chlorin
m-THPP	5,10,15,20-Tetrakis(*meso*-hydroxyphenyl)porphyrin
NLS	Nuclear localization signal
PAMAM	Poly(amidoamine)
Pc4	Silicon phthalocyanine 4
PCL	Polycaprolactone
PDT	Photodynamic therapy
PE	Phosphatidylethanolamine
PEG	Poly(ethylene glycol)
PLA	Polylactide
PLGA	Poly(D,L-lactide-*co*-glycolide)
PMB	Photodynamic molecular beacons
PPI	Polypropylene imine
RES	Reticuloendothelial system
SCC	Squamous cell carcinoma
siRNA	Small interfering RNA
SLN	Solid lipid nanoparticles
SnCe6(ED)	Tin(IV) chlorin e6 monoetylene diamine
SWNT	Single-walled carbon nanotubes
TF	Tissue factor
$TMPyP_4$	*meso*-5,10,15,20-Tetrakis-(*N*-methyl-4-pyridyl)porphine
TPC	5-(4-Carboxyphenyl)-10,15,20-triphenyl chlorin
TPDT	Targeted photodynamic therapy
VEC	Vascular endothelial cells
VEGF	Vascular endothelial growth factor
VP	Verteporfin

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