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Review

Recent progress in tumor photodynamic immunotherapy

Lijun Shen[a,1], Tianjiao Zhou[a,1], Yatong Fan[a], Xin Chang[a], Yi Wang[a], Jianguo Sun[e,*], Lei Xing[a,b,c,d,*], Hulin Jiang[a,b,c,d,*]

[a] State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University, Nanjing 210009, China
[b] Jiangsu Key Laboratory of Druggability of Biopharmaceuticals, China Pharmaceutical University, Nanjing 210009, China
[c] Jiangsu Key Laboratory of Drug Screening, China Pharmaceutical University, Nanjing 210009, China
[d] Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, China Pharmaceutical University, Nanjing 210009, China
[e] Key Lab of Drug Metabolism and Pharmacokinetics, State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, China

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ABSTRACT

Photodynamic therapy (PDT) is a promising alternative approach for effective cancer treatment, which can directly destroy local tumor cells due to the generation of cytotoxic singlet oxygen and reactive oxygen species (ROS) in the tumor cells. Intriguingly, PDT-mediated cell death is also associated with anti-tumor immune response. However, immunosuppression of tumor microenvironment is able to limit the immune response induced by PDT, it is therefore necessary to combine with immunocheckpoint inhibitor and immunoadjuvant for synergistic treatment of tumors. Herein, the recent advances of PDT, immunotherapy, and photodynamic immunotherapy are reviewed

1. Introduction

Immunotherapies have received a lot of attentions and have made rapid clinical progress over the past few years, which can be used for many purposes, like the treatment or prevention of diseases [1]. Nowadays, it is a powerful clinical reality for many diseases, with a steady progression of new drug approvals and a massive pipeline of additional treatments in clinical and preclinical development [2]. Different from other treatments, immunotherapeutic targets are not the cells or tissues in the lesion, but the body's own immune system, which are artificially enhanced or suppressed to intervene diseases. The beginning of immunotherapy was the use of vaccine, which was originally used for livestock [3]. Since then, researchers have used immunotherapeutic agents for the treatment of human diseases, such as heart diseases [4], tuberculosis [5], epidemic typhus [6]. Simply, immunotherapy treats the disease by enhancing or suppressing the body's immune system through chemical or biological immunomodulators. There are plenty of evidences manifesting that the host immune system is able to recognize and react against malignant cells, which illustrates the therapeutic possibilities of anticancer immunotherapy [7,8]. Since the early efforts that take advantage of the immune system to treat cancer have pioneered by Dr. William B. Coley [9], great advances were made in the field of cancer immunotherapy, from cancer immunosurveillance [10] to cancer immune-edit [11]. What is more, tumor genomics and host immune responses are closely related, which means cancer immunotherapy have significant influence on cancer patients [12]. However, of note is the fact that immunotherapy for cancer treatment alone is not efficient enough to eradicate various tumors as expected. Hence, it calls for a new strategy to develop combination therapy to overcome the shortcomings of immunotherapy.

In order to acquire efficient therapeutic effects, photodynamic therapy (PDT) is becoming a hot research field, which can directly kill the cells after irradiation [13]. In the research of PDT, it was found that the immune response triggered by PDT treatment played a crucial role in inhibiting tumor recurrence [14]. This suggests a link between PDT and immunotherapy, which may improve the treatment efficiency, and enhance the cure rate of diseases. PDT can induce inflammation at the treated site, then massive invasion of activated myeloid cells and generate significant effect on the immune system [15]. For tumor treatment, PDT damage cancer cells and promote the release of tumor antigen to induce acute inflammatory response, achieve leukocyte infiltration [16] and further activate lymphoid cells, thereby leading to tumor-specific immunity [17], the activation of an immune response including the innate and adaptive immune response [13]. The nature, rate, and extent of cell death induced by PDT are of vital importance in determining the generation of effective anti-disease

* Corresponding authors.
E-mail addresses: jgsun@cpu.edu.cn (J. Sun), xinglei6xl@163.com (L. Xing), jianghulin3@163.com (H. Jiang).
[1] These authors contributed to this work.

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immune response [18]. In addition to the direct immune stimulation by PDT, the combination of immune-related adjuvants can further enhance the body's immune response and increase the immune killing effect [19].

Given that PDT can be effectively combined with immunotherapy, and a large number of researches have emerged in the field of combined application and have made gratifying progresses in recent years, herein we reviewed the combined application of PDT and immunotherapy. With PDT as the main line, the progress of PDT which can directly induce immune response and enhance therapeutic effect is overviewed, and the application of immunotherapy combined with immunoadjuvant is also summarized.

2. Photodynamic therapy

PDT has been used to treat diseases for more than three thousand years, which can be verified in the history of ancient Egypt, ancient India and China [20]. Generally speaking, light therapy originated in Denmark at the end of the 19th century, Finsen first utilized red light to treat smallpox pustules and prevent suppuration, which was the beginning of the modern light therapy [21]. By 1900s, Raab found the cytotoxicity of acridine in the presence of certain wavelengths of light, confirming that wonderful combination of light and certain chemicals could induce cell death [22]. Three years later, Herman treated skin cancer by topical administration of eosin and white light [23]; until 1906, Noguchi presented a new concept of "photodynamic action" for the first time [24]. In the next hundred years, a myriad of researchers found that porphyrins are phototoxic, and successively synthesized a variety of porphyrin derivatives and clarified the principle of "photodynamic action". Until 1999, Porfimer sodium, the first PDT drug in the world, was approved in Canada [25]. Since then, PDT has become a routine treatment widely used in the clinical setting, due to its high selectivity, low invasiveness, reproducibility, low toxicity and protection of important organs and appearances. It is mostly used for the treatment of benign tumors in the superficial layer and the adjuvant treatment of malignant tumors.

2.1. Three elements of PDT

Admittedly, PDT consists of three elements: light, photosensitizer (PS) and oxygen. The overall process of PDT is as follows: When exposed to specific wavelengths of light, PS becomes activated and converts the light energy into chemical energy, which is then transferred to oxygen to generate a large amount of ROS, such as singlet oxygen and free radicals. These ROS induce apoptosis or promote cell death by oxidizing lipids, proteins and nucleic acids and thereby causing oxidative damage to cells [26,27]. Based on these unique attributes, none of these three components is individually toxic. Namely, light, PS and oxygen are indispensable and directly affect the efficiency of PDT.

2.1.1. Specific wavelengths of light

Depending on the traits of the PS, visible and near-infrared light are able to be used preferentially to trigger PDT [28]. Along this line, the maximum excitation wavelength of PS is generally chosen as the irradiation wavelength to maximize the energy conversion efficiency. The longer the wavelength of light is, the stronger the light transmittance is [29,30]. Numerous studies suggests that near-infrared light, such as 660 nm, is able to penetrate tissue till 2–3 mm; when extend the wavelength to 700–800 nm (near-infrared I band), its penetration depth increases to 5–6 mm; when extend the wavelength to 1000–1700 nm (near-infrared II band), its penetration depth increases to 10 mm accordingly [31,32]. To this end, the optimal wavelength needs to be chosen for the purpose of deeper tissue irradiation.

2.1.2. PS

PS, also known as sensitizers, photocrosslinkers, are capable of transferring light energy to chemical energy for light-insensitive substrate such as O_2 [33]. Usually, an ideal PS agent should have following traits: (1) stable enough at both room temperature and under biophysical conditions for convenient storage; (2) high conversion efficiency together with no dark toxicity; (3) low manufacturing costs and commercially available [34]. Traditional PS possess many defects, such as complex components, poor solubility and stability, which have significant influence on its clinical application. To surmount these problems, increasing numbers of researchers focus on finding and synthesizing new types of PS, such as inorganic PS [35–37], metal-organic composite PS [38–40] and radiation-exciting PS [41], which have more single structure, higher active oxygen production and conversion efficiency. The increase in the variety of PS and the improvement of function vigorously promote the development and application of PDT.

2.1.3. Oxygen

Oxygen generally refers to the oxygen content of the diseased region in PDT. A myriad of tumor treatments, such as chemotherapy and radiation therapy, are ineffective due to its low tumor oxygenation levels. The same is true for PDT, on account of the fact that the direct killing effect in PDT is caused by active oxygen, ROS molecules. The higher oxygen concentration is, the stronger killing effects on tumor cells are [42]. Therefore, oxygen concentration in the lesion region directly determines the speed and extent of the PDT.

2.2. The mechanism of PDT

In simple terms, the photosensitizer molecule is in a singlet ground state (S_0) with two electrons rotating in opposite directions. Following the absorption of light energy under specific wavelength excitation, the PS is activated to reach an excited singlet state (S_1). The S_1 state is stabilized either by emitting fluorescence or by a non-radiative process *via* intersystem crossing from a singlet to a triplet state (T_1). The T_1 state PS is able to be maintained for a longer period of time and undergo two kinds of reactions, namely type I and type II reactions [43,44] (Fig. 1). For one thing, it can directly react with surrounding substrates such as the cell membrane or other biomolecules to form radicals and radical ions (type I reaction); for another, the activated PS transfer its energy directly to oxygen to produce singlet oxygen-a highly cytotoxic ROS (type II reaction). Of note, both type I and type II reactions occur simultaneously and the ratio between these processes is depend on the virtue of the PS and the concentrations of O_2 and other substrates [26,34,45].

Due to the lack of active oxygen depletion system in tumors, singlet oxygen generated by PDT can effectively oxidize biological substances such as lipids, proteins and nucleic acids in cells,

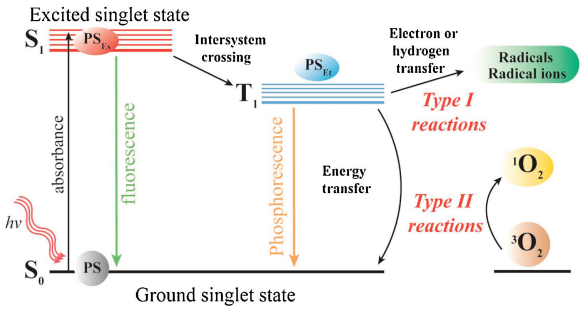

Fig. 1. Molecular mechanism of PDT.

destroy the normal physiological structure of cells, disturb cell metabolism, and finally induce cell necrosis and apoptosis [46]. This is one of the most important PDT mechanisms currently accepted by researchers. In addition, PDT is also capable of destroying vasculature in tumor tissues, leading to ischemia, hypoxia, and insufficient supply of nutrients to induce cell death [47,48]. Last but not least, after light irradiation, it also induce rapid infiltration of various immune cells to tumor sites, activate the complement system, and promote the release of various cytokines/chemical factors to initiates immune response to kill residual tumor cells [49,50]. These three mechanisms occur simultaneously and influence each other in PDT. As a consequence, the combination of PDT and immunotherapy opens up new perspective for overcoming the clinical challenges of PDT and improving therapeutic effect.

3. Cancer immunotherapy

The cancer is a genetic disease and its growth is accompanied with accumulation of mutations, which accelerates their evolutionary fitness to different attacks [51]. However, it is the evolution that makes the cancer cells diverges from normal cells, making them to be recognized as alien invaders by the host immune system. The debate about whether the immune system can destroy tumors has lasted for nearly a century. Until now, many preclinical and clinical inspiring evidences showed that immunotherapy is proving to be an effective therapeutic approach in a variety of cancers [52]. In this section, we will discuss about the pathway of tumor immune response and the challenges of tumor immunotherapy.

3.1. The pathway of immune response

The whole cancer immune response is continuous and dynamic [53]. In the ideal conditions, the process begins with tumor antigen release, which can be derived from exogenous delivery such as therapeutic vaccine or factors releasing from apoptosis and/or necrosis tumor cells. This antigen will show “dangerous” signals and activate innate system. The activated cells such as B cells and NK cells of the innate immune system are not only responsible for surveying and informing, but also have intrinsic antitumor functions, including lysis of tumor cells and production of cytokines that inhibit tumor growth or block angiogenesis. Simultaneously, the tumor-derived antigens can also stimulate professional antigen-presenting cells (APCs), particularly dendritic cells (DCs), which is the essential part of the immune response. In the draining lymph nodes, the DCs process the antigens for presentation or cross-presentation on MHC class II and class I molecules to T cells. And the antigen-educated T cells (along with B cells and NK cells) will exit the lymph node and migrate to the tumor microenvironment, infiltrate tumor tissues and kill the tumor cells [54,55] (Fig. 2).

3.2. Challenges in cancer immunotherapy

Immunotherapy has been studied in preclinical and utilized in clinical, such as Herceptin monoclonal antibody, bevacizumab and chimeric antigen receptor T-cell immunotherapy (CAR-T). Although immunotherapy has become a promising treatment, many evidences indicate that the immune system is progressively unable to recognize cancer, therefore the cancers can avoid the attack of immune cells, namely immune resistance or immunosuppress [56] (Fig. 2), which markedly reduces the efficiency of immunotherapy. With the study about the molecular mechanisms of immunotherapy resistance, many factors influencing the cancer immunotherapy has been calculated in

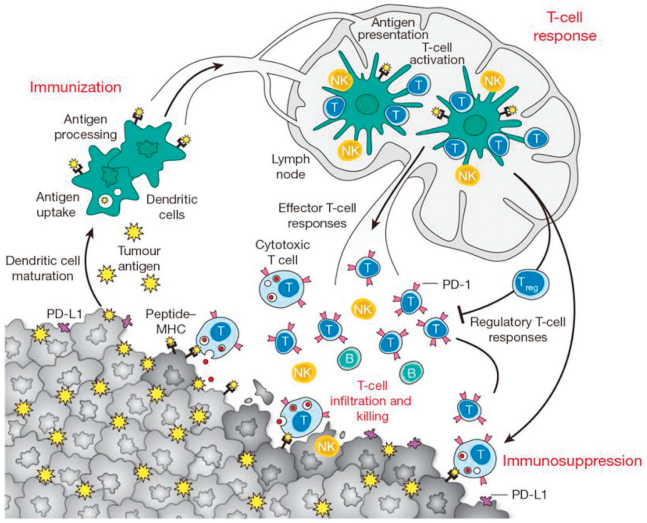

Fig. 2. Generation and regulation of antitumor immunity. Copied with permission [54]. Copyright 2011, Nature.

the Table 1, and additional factors are being discovered rapidly [51].

3.2.1. Checkpoints

Actually, the main part of the immunotherapy is the process of recognizing and killing tumor cells generated by active-T cells. However, because the growth of tumor cells is initiated by mutations, the surviving tumor cells have evolved the ability to deceive the immune cells, making T cells unable to recognize them. This negative regulatory pathway is called checkpoint [57].

Currently the most concerned is cytotoxic T-lymphocyte protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1), a major achievement in 2018 by Nobel Physiology or Medicine, awarded to James and Honjo who found the immune checkpoint, making immunotherapy become a new pillar of cancer treatment [58]. In the process of T cells full activation, it is necessary that the interaction between the costimulatory molecular CD28 and B7, which is closely regulated by inhibitory checkpoints. In 1980s, the CTLA-4 was discovered expressed on regulatory T cells (Tregs) and activated effector T cells [59] and the competition between CTLA-4 and CD28 for B7 ligand leading to the inhibition of proliferation was reported in 1995 [53,60]. Different form CTLA-4, the PD-L1 can be expressed directly on tumor cells, which interacts with the inhibitory receptor on activated T cells named programmed death-1 (PD-1) and prevent tumor cells from immune attack [61]. Thanks to the discoveries, immune checkpoint blockade was applied in clinical and gains great progress in immunotherapy, making the strategy combined with various clinical therapeutics, such as surgery, radiation, chemotherapy and photodynamic/photothermal therapy [62] (Fig. 3).

3.2.2. Tumor hypoxic microenvironment

As we mentioned before, the activated T cells need finally infiltrate into tumors and recognize tumor cells to perform immunotherapy. However, many data suggested that the entire immune process may not always occur completely [63]. As reported by Stephen *et al.*, anti-tumor T cells evaded hypoxic environment and were inhibited extracellular adenosine-rich tumor microenvironment by the A2A adenosine receptor [64]. In addition, it has been reported that hypoxia promotes tumor resistance to immune attack through several HIF-dependent mechanisms, such as increasing the expression of PD-L1 and CTLA-4, upregulating CD47 and enhancing myeloid-derived

Table 1
Factors that influence the cancer-immune set point.[a]

The pathway of immune response	Types of influencing factors	Sources				
		Cancer	Therapeutic agents	Environmental factors	Microbiome	Host genetics
Release of cancer-cell antigens	Positive effect	Neoantigens; Viral antigens; Cancer-associated antigens	/	/	ROS	/
Cancer-antigen presentation	Negative effect	/	HMGCR inhibitors	Excerise-induced IL-6; $1,25(OH)_2D_3$; Photoimmune IL-6 ARNTL	Endotoxin response; TNF-α	TNF-α; NF-κB; FcγRIII; Inflammasome pathway; TLR; NOD2; ATG16L
	Negative or positive effect	/	Traditional Chinese medicine	/	/	/
Priming and activation of T cells	Positive effect	RANKL; Antigen immunogenicity		/	/	TCR
	Negative effect	/	Glucocorticoids; HMGCR inhibitors	$1,25(OH)_2D_3$; Vitamin A; Age-related loss of TCR diversity		
	Negative or positive effect	/	NSAIDs			HLA type
Trafficking of T cells to tumors	/	/	/			
Infitration of T cells into tumors	Positive effect		Anti-VEGF			
	Negative effect	Fas ligand; TGF-β; LOX; VEGF; CAFs/collagen; Chemokines				
Recognition of cancer cells by T cells	Positive effect	Antigen expression	MEKi			
	Negative effect	↓MHC class I expression; ↓TAP-1; ↓B2M; BRAF; KRAS	/			
Killing of cancer cells	Positive effect	/	/			JAK-STAT
	Negative effect	PD-L1; Hypoxia; IDO-1; RANKL	/	Exercise-induced IL-10		

ARNTL: aryl hydrocarbon receptor nuclear translocator-like protein 1; ATG16L: autophagy-related protein 16; FcγRIII: Fc γ receptor III; HMGCR: HMG-CoA reductase; JAK/STAT: Janus kinase-signal transducers and activators of transcription; LOX: lysyl oxidase; NOD: nucleotide-binding oligomerization domain-containing protein; NSAIDs: non-steroidal anti-inflammatory drugs; RANKL: receptor activator of NF-κB ligand.

[a] The factors are placed on rings that denote their type, and each factor is also placed in the step of the cancer-immunity cycle in which they mainly act. For example, variations in HLA type reflect host genetics and are of greatest importance for T cell activation. Additional factors are being discovered rapidly.

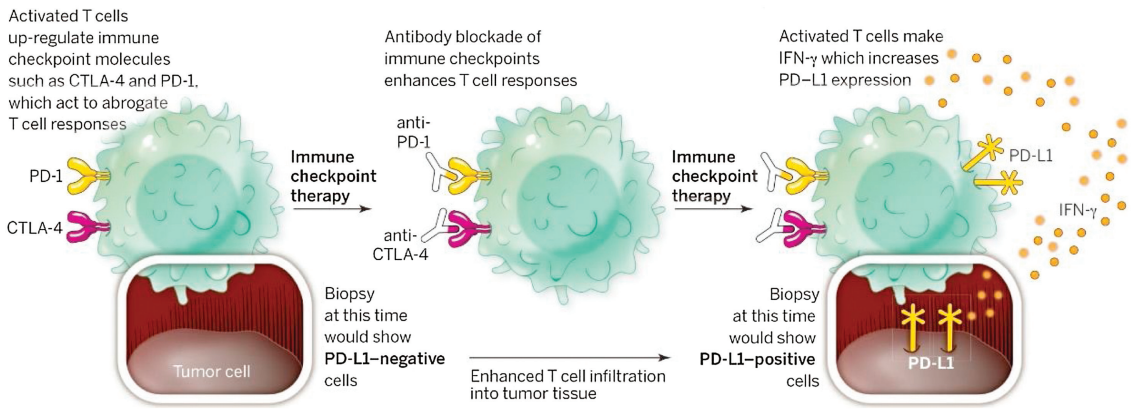

Fig. 3. Blockade of immune checkpoints to enhance T-cell responses. Copied with permission [57]. Copyright 2012, Nature Reviews Cancer.

suppressor cells (MDSCs) [65]. Therefore, many researchers chose to normalize the hypoxia in tumor tissue for the deep infiltration and better efficacy of killing tumor cells generated by activated T cells.

3.2.3. Indoleamine 2,3-dioxygenase (IDO)

IDO is an enzyme catalyzing tryptophan degradation that is constitutively expressed in human tumors and infiltrating myeloid cells. And it seems to block the proliferation of T lymphocytes,

which are extremely sensitive to tryptophan shortage because they have a tryptophan-sensitive checkpoint [66]. Therefore, the same as the CTLA-4 and PD-L1, the IDO is also an important soluble mediator contributing to the peripheral immune tolerance because of the tumoral immune resistance, and nowadays IDO inhibition is an active field of drug development. Recently, the research on IDO inhibitors has attracted extensive attentions, various structural types of IDO inhibitors have been discovered by means of high-throughput screening methods and structure-based drug molecular design methods. Ten small molecule IDO inhibitors have entered the clinical research stage, such as NLG919, Epacadostat (INCB24360), Indoximod, but there are still no drugs listed [67]. It is worthy to mentioned that IDO inhibitor shows a better complement only when the therapy combines with chemotherapy and radiotherapy in the mouse model of glioblastoma [68]. Therefore, many preclinical studies of IDO inhibitor prefer to be combinated with photodynamic therapy [69], radiotherapy [70] and RNAi therapy [71].

3.2.4. Tumor stem cells (tSCs)

tSCs are seed cells for tumorigenesis and metastasis, which possess the ability of self-renew, proliferating and incompletely differentiation. Additionally, increasing evidences have shown that the tSCs can overcome the chemotherapy, radiotherapy and immune attack which might be the root cause of tumor recurrence. With extensive studies about the different forms of resistance, the mechanism might be the ability of potent DNA reparation and the capability of differentiating into matrix and vascular structures which support tumor growth and migration [72]. Besides, it is also reported that the tSCs possess the capability of antigen-editing and maintain only limited antigen presentation, which leads to the immune cells unsuccessfully recognize tSCs [73].

Because there are still many unknowns in the tumor immune response process, more immunosuppressive mechanisms still need to be discovered. Aiming at the common types of immunosuppression which have been found, researchers and physicians have proposed possible strategies to prevent or solve the problem and certain clinical results have been achieved, which make us believe that immunotherapy will gradually become an important tumor treatment.

4. Photodynamic immunotherapy

With the development of science and technology, the role that PDT played in antitumor immunity is gaining more and more attentions in relatively recent times aside from oxidative damage and anti-vascular effect. As described above, there are three mechanisms for PDT to induce immune responses, rapid infiltration of various immune cells, activation of complement system, and release of numerous cytokines, that jointly complete the immune response.

After PDT treatment, cell debris which comes from oxidative damage of tumor cell membranes and cytoplasm acts as inflammatory mediators which are involved in inducing acute inflammatory reaction [15,17,74,75] (Fig. 4). Under such circumstances, a multitude of immune cells, such as neutrophils [76], mast cells [77], and monocytes/macrophages [78] are rapidly accumulated in the treated sites, which then release various cytokines and chemokines to kill tumor cells. In addition, pro-inflammatory cytokines lead to activation of complement cascade and then recruit a large number of neutrophils, macrophages and other immune cells with complement receptors [79,80]. Aside from inflammatory mediators, the cell debris can also serve as tumor-associated antigens, which are capable of being presented to T cells. Correspondingly, T cells are able to be activated and

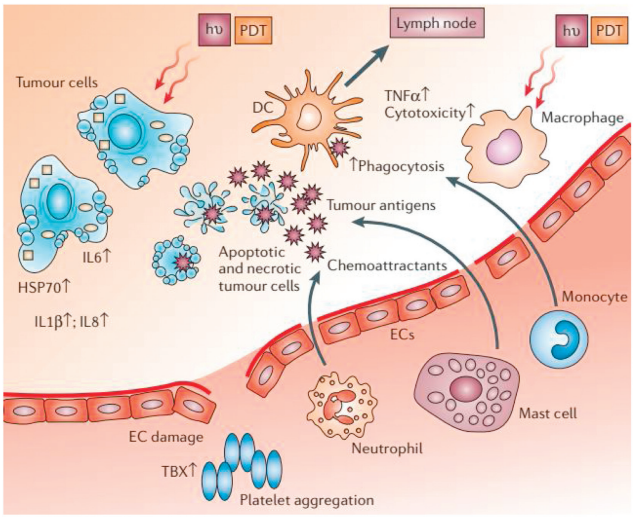

Fig. 4. Inflammation induced by photodynamic therapy. Copied with permission [75]. Copyright 2006, Nature Reviews Cancer.

differentiated into cytotoxic T cells directly bound to MHC class I on the tumor cell surface, thereby lysing target cells [81,82].

As a consequence, tumor ablation induced by PDT is a result from the combined effect of oxidative damage and immune response. Not only can PDT serve as cancer vaccines due to its ability to directly induce immune responses, it also can be used in conjunction with immune-checkpoint inhibitors and other immunoadjuvant to directly or indirectly amplify immune response induced by PDT for cancer treatment.

4.1. PDT-generated cancer vaccines

Since the phenomenon that PDT can trigger anti-tumor immunity has been discovered by Canti *et al.* [83], more and more researchers focus on the exploitation of the immune-activating capacity of PDT [84,85]. A particularly interesting one is PDT-generated vaccines which are the attenuated or killed forms of the tumor cells, similar to conventional vaccines. As reported by Gollnick *et al.*, cancer vaccine, namely cell lysate generated by PDT, could trigger cytotoxic T-cell response unlike other cell lysate generated by UV or ionizing radiation [86]. The distinct advantage of vaccine generated by PDT is specific and powerful for tumor cells, which demonstrates that PDT-derived vaccine has significant clinical potentials for the treatment of various cancers. Based on their research, Korbelik and co-workers sequentially injected squamous cell carcinoma (SCCVII) cells treated by PDT and cyclophosphamide or all-trans retinoic acid (ATRA) for the purpose of finding out the relationship between PDT-generated cancer vaccines and host's immunoregulatory cells [87]. They found that the depletion of Tregs due to the administration of cyclophosphamide and ATRA was conducive to enhance the therapeutic efficacy of PDT vaccine. Hence, it calls for a new strategy to augment tumor immunogenicity of PDT-generated cancer vaccines for the purpose of amplifying immune response.

DCs, one of the most potent APCs in human body, can serve as a vaccine in immunotherapy for cancers but not as effective as expected [88,89]. Therefore, the apoptotic tumor cells produced by PDT are capable of serving as tumor antigens to combine with DCs, thereby overcoming their deficiency and then eradicating tumors. For instance, Zhang and colleagues [90] made use of Deuteporfin and DCs to investigate the anti-tumor and immune efficacy of photodynamic immunotherapy (PIT). *In vivo* animal experiment showed an excellent outcome of slowing down the tumor growth

and prolonging survival time in PDT and PIT group. Additionally, the poor antitumor effect of the DC group was in part attributable to the lack of tumor antigens. Thus, PDT and DCs are mutually reinforcing so that the outstanding antitumor effect can be achieved.

Further researches carried on investigating how PDT had influence on the DC-mediated immunotherapy. Jie *et al.* [91] prepared a DC-based cancer vaccine, named ALA-PDT-DC vaccine, which was constitutive of DCs and 5-aminolevulinic acid (ALA) for the treatment of skin squamous cell carcinoma. When applied to the skin, ALA was immediately converted to its active form protoporphyrin IX, as a photosensitizer to trigger PDT. Intriguingly, the results manifested that the apoptotic tumor cells damaged by PDT could effectually stimulate and enhance the maturations of DCs for prominent immunotherapy, which is far stronger than monotherapy. Very recently, Zhang and co-workers further confirmed that ALA-PDT-DC vaccine could induce immune effects by means of increasing the activity of CD4+ and CD8+ T cells in the tumor [88]. On the basis of these findings, PDT-DC vaccination could be developed as a synergetic therapy to treat various tumors and inhibit metastasis and recurrence.

4.2. PDT combined with immune-checkpoint inhibitors

4.2.1. Anti-PD-L1/Anti-PD-1

Stimulation of the host immune system has been considered to be a promising clinical modality, which generate an antitumor immune response to control tumor growth [92]. However, a self-protecting mechanism utilized by tumor cells may develop against the host immune response [93]. Admittedly, PD-L1 and its receptor PD-1 are two crucial immune checkpoint molecules in the processes of immune resistance [94,95]. Based on the notion, blocking PD-1/PD-L1 interaction is conducive to treat tumor in clinical trial. There are two promising ways to block PD-1/PD-L1 interaction, which is beneficial to amplifying immune response induced by PDT.

For one thing, using PD-L1-targeting small interfering RNA (siRNA) is an effective method to block PD-1/PD-L1 interaction. Wang and colleagues [96] made use of an acid-activatable cationic micelle, photosensitizer, and PD-L1-targeting siRNA to rationally prepare a multifunctional micelleplex, POP-PD-L1 micelleplex. The micelleplex was inert at physiological conditions but dissociated at an acidic microenvironment, which generated ROS and further induced oxidative damage and adaptive immune response by eliciting HSP70 and NF-κB expression and recruiting tumor infiltrating T cells. Furthermore, PDT-induced antitumor immune response was augmented by means of RNA interference to silence PD-L1 expression.

Another example silencing PD-L1 expression was reported by Li's group in 2016 [97]. The author reported a pH-responsive micellar nanocomplex PCPP@MTPP@siPD-L1, which both integrated (PD-L1)-blockade siRNA and mitochondrion-targeting photosensitizer. The micellar nanocomplex was acidity-responsive and size/charge changeable, which was conducive to improve the tumor penetration and cellular uptake under the weakly acidic tumor microenvironment. PS could specifically accumulated in mitochondria to trigger tumor cells apoptosis upon laser irradiation and activate immune response, while the siPD-L1 would effectually silence checkpoint gene PD-L1 expression to suppress immune resistance, thereby augmenting immune response induced by PDT. These findings represented a new paradigm for the combination of PDT and gene therapy to trigger more powerful immune response.

For another, utilizing anti-PD-1 or anti-PD-L1 antibody to block PD-1/PD-L1 interaction also show positive antitumor effects in clinic studies. Gao *et al.* [98] reported a phthalocyanine dye-labeled NIRF probe DSAB-HK to investigate whether PDT and immune checkpoint inhibitor could show synergistic effect for tumor treatment. The results demonstrated that anti-tumor immunity triggered by PDT could be effectively enhanced by PD-1 immune checkpoint inhibitor, both of which could be used for the treatment of primary tumors and metastases.

Along this line, Lan and colleagues [99] also made great efforts on the combination of anti-PD-L1 antibody and PDT. A nanoscale metal-organic framework (nMOF) was rationally designed by means of anti-PD-L1 antibody and Fe-TBP, which converted endogenous H_2O_2 into O_2 to overcome tumor hypoxia through a Fenton-like reaction and improved cancer immunotherapy by significant tumor infiltration of both CD4+ and CD8+ cytotoxic T cells. In the same way, Duan and co-workers [100] reported a nontoxic core-shell Zn-pyrophosphate (ZnP) nanoparticle co-loaded photosensitizer pyrolipid, which directly killed primary tumor cells due to the generation of ROS and indirectly damaged distant tumor cells through innate and adaptive immune response. When combined with anti-PD-L1 antibody, these nanoparticles substantially increased the ability of cancers responding to anti-PD-L1 antibody. Above all, it shows great promise to take advantage of anti-PD-1 or anti-PD-L1 antibody to relieve immunosuppression thereby strengthening immune response.

4.2.2. Anti-CTLA-4

CTLA-4, as a significant immune checkpoint molecule, is able to modulate the immunosuppressive environments within tumors region just like PD-1/PD-L1 [101]. As reported by Kleinovink *et al.* [102], combination therapy of PDT and CTLA-4 blockade excellently improved therapeutic efficacy, survival time of tumor-bearing mice and inhibited tumor metastasis. To gain more insight into the relationship between CTLA-4 and PDT, Xu and colleagues [39] reported upconversion nanoparticles (UCNPs) which co-loaded with Ce6 and imiquimod (R837), a Toll-like-receptor-7 agonist for the integration of PDT and immunotherapy. Obviously, Ce6 was able to destroy tumors under near-infrared (NIR) irradiation as an outstanding initiator of PDT. Synchronously, R837 amplified antitumor immune responses serving as the adjuvant. Based on these unique components, the combination of UCNP-Ce6-R837 and CTLA-4 checkpoint blockade showed excellent efficacy in eradicating tumors, which not only inhibited the growth of primary and distant tumors but also achieved a long-term immune memory function to prevent tumor recurrence and metastasis.

Similarly, Meng *et al.* [103] designed a light-triggered *in situ* gelation system containing photosensitizer-modified catalase (Ce6-CAT) together with poly(ethylene glycol) double acrylate (PEGDA) and immune adjuvant. Under light exposure, *in situ* gelation was produced due to the polymerization of PEGDA, which bring about long-term tumor retention of Ce6-CAT and R837. When further combined with CTLA-4 checkpoint blockade, these gels exerted not only the abscopal effect but also effective long-term immune memory protection for inhibiting tumor metastasis and recurrence.

In relatively recent times, CTLA-4 blockade has already been approved by the U.S. Food and Drug Administration (FDA) for treatment of several types of cancers. Therefore, combination therapy of PDT and CTLA-4 blockade has outstanding value for clinical translation, especially for tumor metastasis.

4.2.3. IDO inhibitors

IDO is also one such checkpoint which prevent the clonal expansion of T cells and facilitate T cell apoptosis by depletion of Trp and production of Kyn [104]. On account of modest effect as monotherapy, it became foremost important to combinational use IDO inhibitors and other therapies for effective antigen presentation and sufficient antitumor immunity. Our previous research

Table 2
Recent development in photodynamic immunotherapy.

	Immunoadjuvant	Immunomodulatory function	References
PDT-generated cancer vaccines	cell lysate generated by PDT	Cytotoxic T-cell response	[83,86]
	SCCVII cells treated by PDT/ cyclophosphamide or ATRA	Cytotoxic T-cell response Depletion of Tregs	[87]
	DCs	Promoting the maturations of DCs/increasing the activity of CD4+ and CD8+ T cells	[88,90,91]
PDT combined with immune-checkpoint inhibitors	PD-L1-targeting siRNA	Suppress immune resistance	[96,97]
	anti-PD-1 antibody	Suppress immune resistance	[98]
	anti-PD-L1 antibody	Significant infiltration of CD4+ and CD8+ cytotoxic T cells	[99,100]
	CTLA-4 checkpoint blockade	Modulating the immunosuppressive environments	[39,102,103]
	IDO inhibitor	Preventing the clonal expansion of T cells and facilitating T cell apoptosis by depletion of Trp and production of Kyn	[105–107]
PDT combined with other immunoadjuvants	GC	\	[108]
	DMXAA	Decreases of TNF-α	[109]
	CpG oligodeoxynuleotide	Priming immature DCs *via* toll like receptor 9	[110–112]

showed that Ce6 mediated PDT-induced immune could be amplified when jointly used with IDO inhibitor NLG919 [105], which is significative for inhibiting the growth of primary and abscopal tumors.

Another example was reported by Lin's group in 2016 [106], the author described a novel chlorin-based nMOF, namely TBC-Hf, co-loaded an IDO inhibitor in the nMOF channels to achieve a powerful photodynamic immunotherapy. Both local and distant tumor inhibition were consistently achieved in mice treated with IDOi@TBC-Hf due to the increased tumor expansions of T cell in the tumor microenvironment.

In the same way, Song and co-workers [107] synthesized a caspase-responsive chimeric peptide which synchronously integrated photosensitizer PpIX with IDO inhibitor 1-methyltryptophan (1-MT) *via* a peptide sequence Asp-Glu-Val-Asp. When exposed in 630 nm light, the PpIX-1MT nanoparticles induced oxidative damage to produce tumor antigens and facilitated the expression of caspase-3 to release 1-MT, which synergistically reinforce the immune system and effectively activate cytotoxic CD8+ T cells for jointly inhibiting both primary tumor and lung metastasis tumor. Recent development in photodynamic immunotherapy was summarized in Table 2 [39,83,86–88,90,91,96–100,102,103,105–112].

4.3. PDT combined with other immunoadjuvants

Aside from enhancing tumor immunogenicity and relieving immunosuppression in tumor microenvironment described above, there is an ocean of immunoadjuvants such as glycated chitosan (GC) [113], complete Freund (CF) adjuvant, and incomplete Freund (IF) adjuvant [114] with considerable potential for augmenting immune responses. Chen *et al.* made use of several different adjuvants for immunological stimulation to strengthen PDT [108]. The results showed that the immunostimulatory effect of GC is more pleasurable than other commonly used immunoadjuvants, such as CF adjuvant, IF adjuvant, and corynebacterium parvum. In addition, 5,6-dimethylxanthenone-4-acetic acid (DMXAA), as an anti-vascular agent, can exert antitumor effect through the induction of TNF-α. Bellnier and colleagues utilized DMXAA in conjunction with PDT for the treatment of implanted murine RIF-1 tumors [109]. They found that antitumor activity was enhanced due to decreases of TNF-α, when DMXAA was combined with high-dose PDT. All of these findings suggest that the combination therapy of PDT and other immunoadjuvants have far-reaching therapeutic implications.

As mentioned above, R837 was able to amplify antitumor immune responses as a Toll-like receptors (TLR)-7 agonist as a result that TLR are responsible for recognizing antigens and subsequently promoting DC maturation [110]. In addition to R837, CpG oligodeoxynuleotide had also been confirmed to be effective to DC immunotherapy [111,115]. Xia and colleagues [112] used liposomal benzoporphyrin derivative as a PS in combination with immunoadjuvants CpG oligodeoxynuleotide to treat orthotopic metastatic 4T1 breast tumors. They found that improved local tumor control and a survival advantage, which demonstrated that CpG can be a valuable DC targeted immunoadjuvant to combine with PDT for amplified immune response.

5. Perspective

Nowadays, immunotherapy has become an effective means of cancer treatment after nearly a century debate. Accompanied with that, PDT has been successfully used in clinical treatment with the development during 30 years, and has become an alternative treatment to surgery in some cancer therapy. Since the release of antigen and cytokines as well as the evocation of local inflammatory reaction, PDT can be well utilized in immunotherapy, acting as the vaccination. With the deepening of research and clinical data feedback, PDT can indeed successfully evoke an immune response, increase the number of CD8+ T cells in blood, and even inhibit metastasis. However, many evidences have shown that the intense PDT always causes significant up-regulation of IFN-γ, which will increase the up-regulation of PD-1, CTLA-4 and IDO, thereby inhibiting T cell function and induce immune evasion. To address these problems and increase the efficacy, researchers utilize immunological checkpoint inhibitors combining with PDT, which showed potential prospects in clinical. Although the combination therapy of PDT and immunotherapy in clinical application is less, we confidently hope that the immune therapy generated by PDT and the combination therapy will become a successful component in the future cancer therapies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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