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REVIEW

The effect of photodynamic therapy on tumor angiogenesis

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Abstract Photodynamic therapy (PDT), the activation of a photosensitive drug in tumor tissue with light of specific wavelength, has been used effectively to treat certain solid tumors. Though therapeutic responses are encouraging, PDT-mediated oxidative stress can act as an angiogenic switch that ultimately leads to neovascularization and tumor recurrence. This article explores the effect of PDT on angiogenesis in different tumor models. Overexpression of proangiogenic vascular endothelial growth factor, cyclooxygenase-2 and matrix metalloproteases has often been reported post-illumination. Recent clinical studies have demonstrated that inhibiting angiogenesis after chemotherapy and radiotherapy is an attractive and valuable approach to cancer treatment. In this review, we report the effective therapeutic strategy of combining angiogenesis inhibitors with PDT to control and treat tumors.

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Introduction

Photodynamic therapy (PDT) is an oxygen-mediated minimally invasive therapeutic modality. It involves the administration of a tumor-localizing photosensitizer that is subsequently activated with light of specific wavelength thus causing highly selective photodynamic destruction of tumor cells. The evident advantage of PDT over other conventional cancer treatments such as chemotherapy and radiotherapy is its minimal invasiveness, selective targeting and reduced toxicity that allows repeated treatment [1, 2].

Currently, PDT is being successfully used for the treatment of early lung cancers [3, 4], and in dermatology for the treatment of non-melanoma skin cancers and precancerous diseases [5]. PDT has also been successfully employed to treat early carcinomas of the oral cavity and larynx to preserve normal tissue and improve cure rates [6]. Though therapeutic responses are encouraging, recurrences have been noticed due to incomplete PDT which consequently triggers tumor angiogenesis. Non-homogeneous light distribution, incomplete photosensitizer dosage and tissue/tumor dynamics are some of the factors that impose constraints on the efficacy of PDT. Moreover, as PDT-induced oxidative stress can cause hypoxic condition in the surviving tumor cells, it can elicit the expression of angiogenic growth factors and cytokines as an adaptive response [7, 8]. Not only in PDT, hypoxia is also known to reduce tumor sensitivity to radiation therapy and chemotherapy that is associated with decreased local tumor control [9, 10]. Hypoxia induced inflammatory responses,

in turn, can possibly diminish the treatment efficacy by promoting signaling cascades, such as the hypoxia-signaling pathway and vascular endothelial growth factor (VEGF), which initiate angiogenesis.

The development of new blood vessels from preexisting vessels is an important process in tumor progression. It is also well known that tumors require blood vessels that supply oxygen and nutrients to reach metastatic potential [11]. Therefore combining angiogenic inhibitors with chemotherapy and radiotherapy has demonstrated their potential to be an attractive and effective approach to cancer treatment. As a result it has been hypothesized that the development of agents targeting tumor angiogenesis could be an effective strategy to control and treat various malignancies.

The relationship between PDT and angiogenesis was first established by Ferrario and colleagues [12]. They demonstrated that PDT effectiveness could be enhanced by combining angiogenic inhibitors with the treatment regimen. This review article will focus on the effect of PDT on tumor vasculature and the numerous angiogenic growth factors that are overexpressed and play a major role in tumor recurrence after PDT. We will also discuss the angiogenesis inhibitors that have been successfully used in combination with PDT to increase tumor control in different tumor models.

PDT induced vascular damage

Tumor growth depends on functional vasculature for the supply of oxygen and nutrients. Therefore, selectively damaging existing microvasculature and preventing the formation of new blood vessels would improve treatment efficacy. PDT has been known to cause microvasculature collapse leading to severe tissue hypoxia [13]. The vascular effects of PDT can differ greatly based on the different photosensitizers and the drug-light interval administered.

PDT with Photofrin leads to vessel constriction and thrombus formation [14], certain phthalocyanine derivatives cause vascular leakage [15] and mono-L-aspartyl chlorin e6 (NPe6) results in blood flow stasis [16]. Fingar et al. [17] defined the effects of Photofrin PDT on microvasculature in rat cremaster muscle with regard to changes in vessel constriction, vessel leakage and leukocyte adherence. It was reported that higher Photofrin dosage causes vessel constriction and changes in permeability during PDT. Vasoconstriction and complete stasis were observed by Star et al. [18] in hematoporphyrin derivative PDT of tumors, and it was concluded that tumor cell death after photoradiation occurs secondary to destruction of the microvasculature. Henderson et al. [19] reported that both mammary carcinoma (EMT-6) and radiation-induced fibrosarcoma (RIF) tumors responded to PDT with severe vascular disruption, and observed oxygen deprivation in RIF tumors in another study [20]. Vascular damage and blood flow stasis were noticed in tumors treated 5 min after a benzoporphyrin derivative (BPD) injection, leading to long-term tumor regression [21]. PDT after a single dose of the photosensitizer MV6401 induced blood flow stasis [22] and thrombus formation that caused long-term vascular shutdown [23]. Morphological changes after hematoporphyrin derivative PDT included absence of the endocapillary layer and mitochondrial degeneration. It has also been reported that changes in tumor cells followed the changes within the microvasculature, indicating the importance of vascular targeting in PDT [24]. Chen et al. [25] observed increased vascular permeability and blood cell adherence to vessel wall shortly after verteporfin-mediated PDT, further suggesting that the photosensitized vascular permeabilization can be used to improve tumor drug delivery and enhance the therapeutic effect. Although microvascular damage and hypoxia after PDT contributes to greater tumor response, reduction in oxygen during treatment can limit tumor control by inducing the production of proangiogenic markers such as VEGF, cyclooxygenase-2 (COX-2), metalloproteinases (MMPs) and other cytokines, creating enhanced environment for tumor recurrence.

Vascular endothelial growth factor

Vascular endothelial growth factor is one of the most important regulators of angiogenesis that acts as a switch to trigger tumor recurrence by promoting proliferation, migration and tube formation of endothelial cells. Moreover, VEGF binds to the tyrosine kinase receptors VEGFR-1 and VEGFR-2, thus initiating a downstream signaling cascade that promotes angiogenesis [26]. Several groups including ours have reported the upregulation of VEGF following PDT [27–30]. Ferrario et al. [12] revealed that PDT-mediated hypoxia and oxidative stress could be involved in Photofrin-mediated PDT induced expression of hypoxia-inducible factor-1alpha (HIF-1α) and also increased protein levels of the HIF-1 target gene VEGF, in transplanted mouse mammary carcinoma. In a similar study, the same group also reported significant overexpression of HIF-1α and VEGF after Photofrin-mediated PDT in a xenograft model of Kaposi's sarcoma [28]. Increased expression of VEGF was noticed from 0 to 6 h in tumors treated with haematoporphyrin PDT compared to control tumor in a mouse squamous cell carcinoma model [29]. Similar observations were noted by Jiang et al. [31] whereby VEGF levels significantly increased after Photofrin PDT in intracranial glioblastoma xenografts. In an

earlier study, the same research group had reported increased VEGF levels in normal rat brain that induced the formation of aberrant new vessels following treatment with high dose PDT [32]. In another study it was demonstrated that low dose PDT increases endothelial cell proliferation and VEGF expression in nude mice brain [33]. In addition, the upregulation of VEGF in Photofrin-mediated PDT was also observed in the brain tissue adjacent to tumor in a dose dependent manner [34]. Solban et al. [35] investigated the effect of subcurative PDT using BPD as the photosensitizer in an in vivo orthotopic model of human prostate cancer that demonstrated increased VEGF secretion 24 h following PDT and suggested vascular damage and/or a direct effect of BPD to be responsible for this increase. Kosharskyy et al. [36] observed increases in not only VEGF secretion but also incidences of lymph node metastases after subcurative PDT in an orthotopic model of prostate cancer (LNCaP) that created conditions favorable for enhanced tumor growth and metastasis. The same group also investigated the use of an optical molecular imaging strategy to monitor VEGF expression in vivo and effectively labeled and imaged bound VEGF released from the extracellular matrix in response to PDT [37]. Our group has observed increased secretion of HIF-1α and its target gene VEGF in hypericin-mediated PDT in both nasopharyngeal and bladder tumors [27, 38]. Moreover, cellular-mediated long drug-light interval (DLI) hypericin PDT induced greater expression of pro-angiogenic growth factors compared to vascular-mediated short DLI PDT in a bladder carcinoma xenograft model [39]. Zhou et al. [40] demonstrated that the expression of HIF-1α and VEGF increased in PDT-treated tumor samples collected 24 h post-PDT in a mouse model of human nasopharyngeal carcinoma. NPe6 PDT of cytokine-overexpressing Lewis lung carcinoma (LLC/IL-2) tumors revealed that the expression of GADD-5alpha and VEGF are induced after PDT and in particular the expression levels were much higher as compared with those in LLC tumors, 12 h after PDT [41]. However, the application of ALA-PDT resulted in a lowered rate of metastatic spreading and decreased VEGF level in blood serum of 3LL-bearing mice that has been attributed to vascularization disturbances in tumor tissue [42]. Hypocrellin-mediated PDT in human brain tumor cells induced expression of proangiogenic VEGF and of antiangiogenic SFH-1, angiostatin, p43, allograft inflammatory factor-1 and connective tissue growth factor suggesting favorable and deleterious effects of hypocrellin PDT on tumor outgrowth [43]. Based on the above studies, one can infer that PDT using photosensitizers i.e., Photofrin, hypericin, hypocrellins and chlorin e6 increases VEGF concentrations within the tumor tissue and acts as a key regulator of angiogenesis and tumor recurrence post-treatment.

Cyclooxygenase-2

Cyclooxygenase-2 is a rate-limiting enzyme converting arachidonic acid to prostaglandins. It is an important mediator of angiogenesis and tumor growth, is expressed in a wide range of preneoplastic and malignant conditions, and is known to localize in the neoplastic and endothelial cells within the tumors. COX-2 induces angiogenesis mainly by the production of VEGF and by promoting vascular sprouting, migration and tube formation [44, 45]. Ferrario et al. [46] investigated PDT-mediated activation of COX-2 expression and observed prolonged expression of COX-2 protein in treated mouse sarcoma and RIF tumors. Moreover, both porphyrin- and chlorin-based photosensitizers have been reported to elicit PDT-mediated COX-2 expression. In their recent work, Luna et al. [47] demonstrated that multiple protein kinase cascades can be activated by PDT-induced oxidative stress and that the p38 signaling pathway and cyclic-AMP response element 2 (CRE-2) binding are involved in COX-2 expression following Photofrin-mediated PDT. Increased expression of COX-2 mRNA was clearly evident within 30 min of treatment and remained elevated for 12 h post-Photofrin PDT. Also, COX-2 protein expression was upregulated and remained elevated for 72 h. Hendrickx et al. [48] have shown that hypericin-mediated PDT on bladder cells leads to a rapid rise in the cytosolic calcium concentration which is followed by the generation of arachidonic acid by phospholipase A2 (PLA2). The authors demonstrated that inhibition of PLA2 significantly protected cells from the PDT-induced intrinsic apoptosis and thus attenuated the activation of p38 mitogen-activated protein kinase (MAPK) that mediates the up-regulation of COX-2. They further concluded that hypericin PDT of human cancer cells leads to up-regulation of the inducible COX-2 and the subsequent release of prostaglandin 2 (PGE_2). In our earlier study, COX-2 expression at mRNA level was upregulated at 24 h post-hypericin PDT in CNE-2 xenograft tumors compared to controls [40]. Another study that focused on the molecular mechanisms regulating COX-2 expression after low-dose PDT in HeLa and T24 cancer cell line reported PDT induced COX-2 expression in these cells. This expression was attributed to nuclear factor kappa B (NF-κB)-dependent transcription of COX-2 gene without any post-transcriptional regulation, thereby promoting cancer cell regrowth following PDT [49]. To summarize, recent studies have demonstrated that porphyrin and chlorin-e6 PDT induce the expression of COX-2 and MAPK, and NF-κB pathway activates COX-2 expression in hypericin-mediated PDT.

Matrix metalloproteinases

Increasing evidence indicates that the matrix metalloproteinase (MMP) family of extracellular proteinases has an

important role in cancer invasion and metastasis due to its ability to degrade all components of the extracellular matrix. Therefore, MMPs play an important role in maintaining the tumor micro-environment and promoting tumor growth [50]. PDT using photosensitizers Photofrin and hypericin has been shown to upregulate the expression of MMPs. Ferrario et al. [51] documented extensive infiltration of MMP-9 expressing inflammatory cells within the treated tumor and increased protein expression of MMP-9 in mouse mammary carcinoma tumor 24 h after Photofrin-mediated PDT. Similar elevations of MMP-1, MMP-3, and MMP-8 were also observed in tumor tissue treated with PDT. On the other hand, our group has reported down-regulation of MMP-9 expression via inhibition of GM-CSF production, which in turn modulates AP1/NF-κB transcriptional activities in CNE2 cells after hypericin PDT [52]. An increase in MMP-1 mRNA and protein expression has also been documented in well differentiated HK1 and poorly differentiated CNE-2 nasopharyngeal carcinoma cells after hypericin-mediated PDT [53]. A study using 5-ALA PDT in Walker carcinosarcoma model revealed an increased activity of MMP-2 suggesting that MMPs activation and reactive oxygen species are involved in PDT effects [54]. On the contrary, Hexvix (ALA-H)-mediated PDT in medulloblastoma cell line demonstrated downregulation of MMP-2, however MMP-9 expression remained unchanged [55]. In summary, MMP-1, -3, -8 and -9 upregulation have been noticed in Photofrin-mediated PDT and overexpression of MMP-2 was observed post-5-ALA PDT. However, hypericin PDT attenuated MMP-9 and ALA-H-mediated PDT seemed to suppress MMP-2 concentrations.

Other cytokines

PDT-induced inflammatory changes are characterized by enhanced expression of numerous proinflammatory cytokines that stimulate angiogenic processes. Kaposi's sarcoma treated with Photofrin-mediated PDT increased the expression of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) that act as angiogenic regulators [28]. Photochlor-sensitized PDT in combination with 5,6-dimethylxanthenone-4-acetic acid (DMXAA), a vascular disrupting agent, increased the induction of TNF-α and IL-6, at 4 h post-treatment; IL-6 induced the expression of VEGF [56]. NPe6 PDT-induced TNF-α was detected in media of cultured TNF-S2 cells 24 h after treatment [57]. Up-regulation of IL-6 was observed in Photofrin-mediated PDT of cervical (HeLa) and EMT6 cells [58]. In our earlier study we demonstrated that IL-6 transcription was significantly upregulated in hypericin-PDT treated poorly differentiated cells (CNE-2) but not in well differentiated cancer cells (HK1) [59]. On the other hand, PDT caused attenuation of IL-6 in ALA-PDT treated cells. This altered cytokine responsiveness is predicted to affect functions of both normal and tumor cells in the post-PDT tissue environment and may determine the treatment outcomes in patients undergoing PDT [60]. In a study conducted to generate anti-tumor vaccine, PDT-generated lysates were able to activate dendritic cells to express IL-12, which plays an important role in the development of cellular immune response [61]. Bellnier et al. [62] investigated the activity of DMXAA as a modifier of Photofrin-based PDT on implanted murine RIF-1 tumors. The combination of DMXAA and PDT led to a reduction in tumor volume and significant delays in tumor regrowth. DMXAA by itself induced TNF-α in RIF-1 tumors whereas PDT did not. However, the addition of PDT after DMXAA resulted in decreased TNF-α concentration, suggesting that the enhanced antitumor activity of the combination therapy was not attributable simply to an increased induction of the cytokine by PDT over that from DMXAA alone. Ji et al. [63] had reported that high expression of HIF-1α induced by $CoCl_2$ plays an important role in the resistance of Het-1A cells to ALA-PDT. The findings of this study suggested that hypoxia-induced HIF-1α overexpression attenuates PDT efficacy not only by angiogenesis, but also through cellular resistance. PDT-treated nasopharyngeal carcinoma tumors collected at 24 h post-PDT exhibited greater expression of HIF-1α and basic fibroblast growth factor (bFGF) genes compared to control tumors [40]. In our recent study, increased expression of TNF-α, bFGF and interferon-α and -γ (IFN-α, -γ) was observed in long DLI hypericin-mediated PDT compared to short DLI PDT at 24 h post-irradiation in bladder tumors. It was also hypothesized that longer DLI PDT that induces greater oxidative stress within the tumors cells could have caused the noted upregulation of the angiogenic markers [39]. Angiogenesis regulators e.g., angiogenin, bFGF, epidermal growth factor (EGF), placental growth factor (PGF), IL-8 and VEGF-D were upregulated in bladder tumors treated with high dose compared to low dose hypericin PDT [38]. Expression of IL-6 was elevated in PDT treated groups compared to IL-8 suggesting its importance in PDT-induced inflammation. Our earlier investigation proved that nasopharyngeal cells maintained in hypoxic condition induce the expression of the PDGF-β gene whereas upregulation of HIF-1α was observed both in hypoxic and normoxic environment [30]. Interestingly, Buzkulok et al. [64] demonstrated that individual components of the PDT process, photosensitizer alone and light alone as well as the complete PDT procedure, could activate the Akt signaling pathway in murine and human breast cancer cells and tumors. Wei et al. [65] demonstrated in epithelial tumor cells that while the action of IL-6 cytokines through their

membrane receptors is attenuated, regulation by IL-6 via trans-signalling is established, thus enhancing PDT.

Combination of PDT and angiogenesis inhibitors

Recent studies have demonstrated that in vivo PDT treatment of carcinomas improved significantly when combined with angiogenesis inhibitors. Moreover, it has been noted that the tumoricidal activity is enhanced without concomitant increases in normal tissue phototoxicity. As angiogenesis promotes tumor growth and progression, antagonizing this angiogenic response can increase therapeutic gain and enhance the efficacy of PDT. Various angiogenesis inhibitors that have been investigated in combination with PDT are listed in Table 1.

Two angiogenic growth factors, VEGF and COX-2, are expressed by many tumors and seem to be important in sustaining tumor growth. Transplantable BA mouse mammary carcinoma treated with PDT and non-specific antiangiogenic peptides, IM862, a dipeptide and EMAP-II, a single chain polypeptide, increased tumor regression by inducing apoptosis and inhibiting VEGF production. However, the anti-angiogenic agents by themselves did not produce the desired outcome [12]. Use of novel antiangiogenic monoclonal antibodies, MF1 and DC101 along with PDT against vascular endothelial growth factor receptors VEGFR-1 and VEGFR-2, respectively, reduced the tumor volume significantly and prolonged the survival time of glioma-implanted animals [31]. PDT followed by administration of an antiangiogenic agent, TNP-470, abolished the increase in VEGF levels caused by subcurative PDT and reduced local tumor growth in an orthotopic model of prostate cancer (LNCaP) [36].

Synthetic RTK inhibitors SU5416 and SU6668 when combined with hypericin PDT significantly extended survival of tumor-bearing host mice [40]. Combining PDT with humanized monoclonal antibody Avastin (bevacizumab) resulted in significant increase in long-term responsiveness of treated Kaposi's sarcoma tumors when compared to monotherapies [28]. Chang et al. [37] used an in vivo optical imaging technique that produces wavelength-resolved fluorescence hyperspectral images to study changes in tumoral VEGF concentration following PDT and Avastin treatment. Their in vivo antigen blocking experiment showed that Avastin pretreatment before imaging blocked the tumoral VEGF and that VEGF-specific contrast agent labeling decreased in proportion to the pretreated Avastin dose, demonstrating that VEGF-specific contrast agent specifically binds to the VEGF protein in vivo. Our group tested the efficacy of Avastin along with hypericin PDT in bladder tumor xenografts. The results demonstrated that the targeted therapy by Avastin along with PDT can improve tumor responsiveness by inhibiting not only VEGF but also other angiogenic proteins i.e., angiogenin, bFGF, EGF, IL-6 and IL-8 [38].

Receptor tyrosine kinase (RTK) inhibitors, PD166285 and PD173074 when combined with hexylether pyropheophorbide-PDT significantly decreased tumor regrowth and displayed potent anti-angiogenic and antitumor activity in a transplanted murine mammary 16c tumor model [66]. Ferrario et al. [46] observed that addition of selective COX-2 inhibitor NS-398 enhanced PDT responsiveness in RIF tumors without increasing toxicity to normal tissue. In a later study the same group investigated COX-2 inhibitors celecoxib and NS-398. Both inhibitors when administered after PDT enhanced treatment efficacy by increasing in vitro apoptosis and decreasing in vivo inflammatory and angiogenic factors. In addition to that, tumor bearing mice treated with the combination therapy exhibited significant improvement in long-term tumor free survival when compared with PDT or COX-2 inhibitor treatments alone. Moreover, administration of celecoxib or NS-398 attenuated tissue levels of prostaglandin E3 and VEGF induced by PDT in treated tumors and also decreased the expression of proinflammatory mediators IL-1β and TNF-α. PDT-treated cells also showed poly(ADP-ribose) polymerase cleavage (PARP) and BCl-2 degradation, which were further enhanced following combined therapy [67]. On the other hand, Makowski et al. [68] reported that neither rofecoxib, NS-398 nor nimesulide were capable of sensitizing C-26 tumor cells to PDT-induced damage. However, their in vivo studies using colon adenocarcinoma model demonstrated that administering nimesulide, a selective COX-2 inhibitor chronically after illumination potentiated antitumor effects of Photofrin PDT leading to complete tumor response in most of the treated mice. In our earlier study, we reported downregulation of COX-2, HIF-1α and VEGF A isomers 165 and 121 when celecoxib was administered 6 h post-PDT in an in vivo nasopharyngeal tumor model [30]. Akita [69] investigated COX-2 expression and the inhibitory effects of nimesulide in combination with ALA-based PDT on human oral squamous cell carcinoma (SCC) cell lines. ALA-based PDT showed an inhibitory effect on two SCC cell lines HSC-2 and HSC-4, however the combination of nimesulide and ALA-based PDT demonstrated a significant synergistic effect on the cellular growth inhibition of only HSC-2, but not of the HSC-4 cell line.

Hendrickx et al. [48] concluded that combination of PDT with pyridinyl imidazole inhibitors of p38 MAPK may improve the therapeutic efficacy of PDT by blocking COX-2 upregulation. Administration of p38 MAPK inhibitor PD169316 abrogated COX-2 expression in PDT-treated cells. NS-398, when administered along with a single dose of NPe6, significantly decreased the weight of colon-38 tumor xenografts; however no effect was noted

Table 1 Angiogenic inhibitors investigated in combination with PDT and their therapeutic outcome

	Tumor model	Photosensitizer	Growth factors investigated	Angiogenesis inhibitor	Findings	Reference
1	BA mouse mammary carcinoma	Photofrin	VEGF, HIF-1α	EMAP-II and IM862	Improved tumoricidal response was observed	Ferrario et al. [12]
2	Intracranial glioblastoma	Photofrin	VEGFR-1 and VEGFR-2	MF1 and DC101	Noted significant inhibition of tumor growth and extended survival of mice	Jiang et al. [31]
3	Prostate tumor	Liposomal BPD	VEGF	TNP-470	Combination treatment abolished the increase in VEGF levels caused by subcurative PDT and reduced local tumor growth	Kosharskyy et al. [36]
4	Nasopharyngeal and bladder carcinoma	Hypericin	VEGF, bFGF, HIF-1α	SU5416 and SU6668	Extended survival of tumor-bearing host mice was noted	Zhou et al. [40]
5	Kaposi's sarcoma	Photofrin	VEGF	Avastin (bevacizumab)	Improved PDT treatment effectiveness	Ferrario and Gomer [28]
6	Prostate and pancreatic carcinoma	Benzoporphyrin derivative	VEGF	Avastin	Successfully monitored treatment-induced changes in VEGF expression using optical molecular imaging system	Chang et al. [37]
7	Bladder carcinoma (MGH)	Hypericin	VEGF	Avastin	Improved tumor responsiveness was observed due to downregulation of VEGF, bFGF and EGF	Bhuvaneswari et al. [38]
8	Murine mammary 16c tumor	Hexylether pyropheophorbide-PDT	Receptor tyrosine kinase	PD166285 and PD173074	Potentiated anti-angiogenic and anti-tumor activity and prolonged the duration of anti-tumor response	Dimitroff et al. [66]
9	RIF tumors	Photofrin	COX-2, VEGF	NS-398	Enhanced PDT responsiveness in RIF tumors without increasing toxicity to normal tissue	Ferrario et al. [46]
10	BA mouse mammary carcinoma	Photofrin	COX-2	Celecoxib and NS-398	Improved in vivo PDT responsiveness by decreasing expression of angiogenic and inflammatory molecules	Ferrario et al. [67]
11	Colon adenocarcinoma	Photofrin	COX-2	Rofecoxib, NS-398 and nimesulide	Administration of nimesulide after illumination potentiated antitumor effects of Photofrin PDT	Makowski et al. [68]
12	Nasopharyngeal carcinoma	Hypericin	COX-2, VEGF and HIF-1α	Celecoxib	Administration of Celecoxib, 6 h post-PDT downregulated proangiogenic growth factors	Yee et al. [30]
13	Oral squamous cell carcinoma	ALA	COX-2	Nimesulide	Combination of ALA-PDT and nimesulide showed inhibitory effect on HSC-2 cells	Akita et al. [69]
14	Urinary bladder and cervix carcinoma	Hypericin	COX-2	p38 MAPK inhibitor PD169316	Improved the therapeutic efficacy of PDT by blocking COX-2 up-regulation	Hendrickx et al. [48]
15	Colon carcinoma	Mono-L-aspartyl chlorine e6	COX-2, IL-2	NS-398	Significantly decreased the weight of colon-38 tumor xenografts	Harvey et al. [70]

Table 1 continued

	Tumor model	Photosensitizer	Growth factors investigated	Angiogenesis inhibitor	Findings	Reference
16	BA mouse mammary carcinoma	Photofrin	MMPs	Prinomastat (AG-3340)	Significantly improved PDT-mediated tumor response	Ferrario et al. [51]
17	Breast cancer tumors	Photofrin	Akt phosphorylation	PI3-K inhibitors	In vitro and in vivo PDT treatments induced Akt phosphorylation. PI3-K inhibitors blocked Akt phosphorylation by increasing apoptosis	Bozkulak et al. [64]

when NS-398 was administered along with fractionated dosing of NPe6-PDT [70]. Moreover, the same study also noted that tumor recurrence was promoted by the enhancement of VEGF expression, mediated by IL-2 upregulation. Therefore, it was speculated that the use of an IL-2 inhibitor may improve the efficacy of NPe6-PDT.

Ferrario et al. [51] showed that Photofrin-mediated PDT induced the expression of MMPs in mouse mammary carcinoma and the adjuvant use of a broad-spectrum MMP inhibitor, Prinomastat (AG-3340) could improve the PDT responsiveness without affecting normal skin toxicity. It was documented that treatment of mouse tumors with PDT induced strong expression of MMP-9 and EMMPRIN (an extracellular MMP inducer) while suppressing TIMP-1 (tissue inhibitors of metalloproteases). In a recent study, the same group also demonstrated that Akt phosphorylation was induced by multiple PDT components in in vitro and in vivo treatments. Additionally, they demonstrated that blockage of Akt phosphorylation using P13-K inhibitors can increase PDT-mediated apoptosis within treated cells [64].

Conclusion

Tumor hypoxia is a therapeutic concern as it reduces the effectiveness of radiotherapy, chemotherapy and PDT, thus leading to angiogenesis and tumor metastasis. Numerous studies have documented the use of anti-angiogenic agents along with conventional cancer treatments to enhance the antitumor response. In a similar way combining angiogenesis inhibitors with PDT in preclinical studies has clearly demonstrated increased therapeutic efficacy. However, blocking one or two molecular pathways might not provide optimal results, as multiple pathways are involved in the migration and proliferation of tumor cells. Therefore, in order to achieve complete and effective tumor response, using combination therapy and targeting different molecular pathways without increasing toxicity, could be an attractive therapeutic strategy.

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