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## Photodynamic Therapy and the Biophysics of the Tumor Microenvironment†

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### Abstract

Targeting the tumor microenvironment (TME) provides opportunities to modulate tumor physiology, enhance the delivery of therapeutic agents, impact immune response, and overcome resistance. Photodynamic therapy (PDT) is a photochemistry-based, non-thermal modality that produces reactive molecular species at the site of light activation and is in the clinic for non-oncologic and oncologic applications. The unique mechanisms, and exquisite spatiotemporal control, inherent to PDT enable selective modulation or destruction of the TME and cancer cells. Mechanical stress plays an important role in tumor growth and survival, with increasing implications for therapy design and drug delivery, but remains understudied in the context of PDT and PDT-based combinations. This review describes pharmacoengineering and bioengineering approaches in PDT to target cellular and non-cellular components of the TME, as well as molecular targets on tumor and tumor-associated cells. Particular emphasis is placed on the role of mechanical stress in the context of targeted PDT regimens, and combinations, for primary and metastatic tumors.

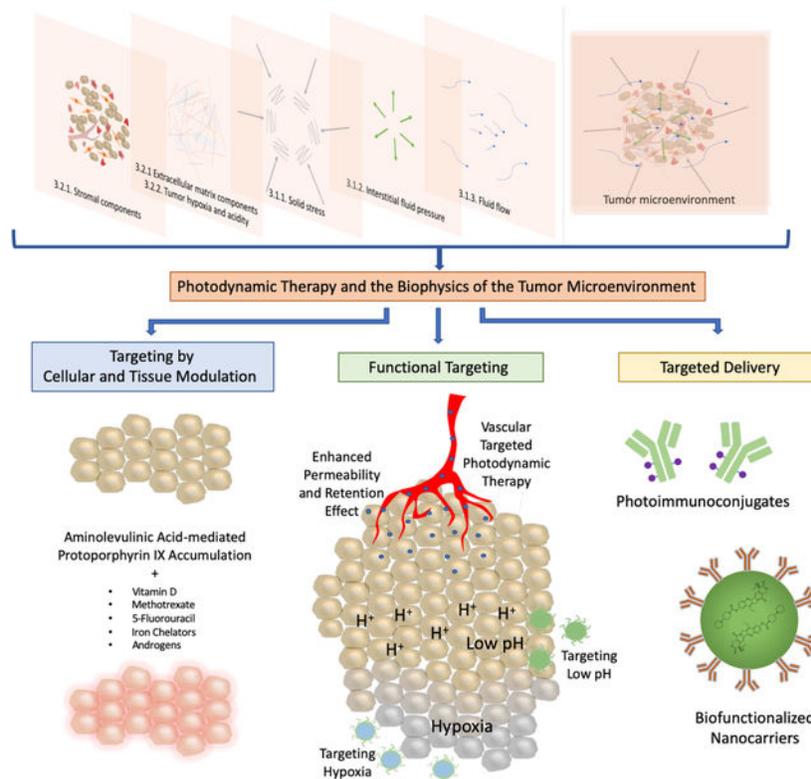
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## Graphical Abstract



Physical stress in the tumor microenvironment impacts actionable targets for photodynamic therapy-based regimens. The design of targeted photodynamic therapy approaches, and rational combinations, that exploit and modulate mechanical stress and stromal components in the tumor microenvironment are discussed in this review.

## INTRODUCTION

Photodynamic therapy (PDT) is a light-based treatment modality that involves electronic excitation of a photosensitizer (PS) to mediate the production of reactive molecular species and induce photodamage at the site of light activation (1–3). PDT provides unique cytotoxic mechanisms and exquisite spatiotemporal control, which makes it an attractive approach to directly target tumor cells and/or the tumor microenvironment (TME, Figure 1). Depending, in part, on the localization of the PS, PDT can directly damage or alter targets in tumor cells such as mitochondrial function, pro-survival pathways, antioxidative effects, efflux transporters, and stimulators of immune response. These topics, and their implications for PDT-based regimens, have been discussed in many excellent articles and reviews (4–11), but are largely outside the scope of the current article. The focus of this review is on physical stress in the TME and implications for the design of targeted PDT approaches and combinations. Major components of the TME are introduced with a focus on mechanical stress. Three categories of targeted PDT that exploit cellular, molecular and mechanical

features in the TME are discussed: I) targeting by cellular and tissue modulation, II) functional targeting and III) targeted delivery (Figure 2).

Targeting by cellular and tissue modulation refers to strategies that manipulate cell metabolism to regulate photosensitizer (PS) production and response to treatment (12). For example, a pro-drug, 5-aminolevulinic acid (5-ALA), can be enzymatically converted into the PS, protoporphyrin (PpIX), through the heme biosynthetic pathway. Much of the research in cellular and tissue modulation to enhance PDT efficacy involves manipulating the rate-limiting steps that convert 5-ALA into heme to increase intracellular PpIX concentrations (12). A recent study examining the effects of matrix stiffness on PpIX production will be discussed (13). Functional targeting refers to approaches that target the unique properties of tumor tissues and the TME including: 1) abnormal vasculature, 2) tumor hypoxia, and 3) increased acidity in tumors. Targeted delivery refers to strategies that involve the use of targeting moieties that facilitate the binding of PS to malignant cells through a molecular recognition process (14). These strategies leverage the overexpression of certain biomarkers on malignant cells that are less expressed on healthy cells to achieve specificity. This review offers a perspective on how these targeted PDT approaches provide opportunities to modulate the cellular and non-cellular components of the TME, with a focus on tumor mechanical properties and survival. The implications for therapy design, taking into consideration the role of mechanical stress in the TME, are discussed.

## THE TUMOR MICROENVIRONMENT AS A TARGET FOR CANCER TREATMENT

### Mechanical Stress in the Tumor Microenvironment

Cells respond to environmental changes by receiving and processing signals that originate in the extracellular space using structures and mechanical linkages among cell surface receptors, the cytoskeleton, and the nucleus (15, 16). Mechanical signals from the extracellular space are sensed by the cells through integrins and other focal adhesion proteins. These signals are transduced through the cytoskeleton and effector signaling cascades to elicit a biological response, and this conversion of mechanical signals to biological responses is termed ‘mechanotransduction’ (17). Many of the components of focal adhesion assembly are also involved in the cell migration process, and mechanical stress can induce migration (18). The tumor cell response to mechanical inputs can also be indirect, as mechanical stresses can influence transport, impeding drug delivery and altering oxygen tension and tumor acidity (19). Tumor cells can be exposed to a variety of mechanical inputs, including solid stress, interstitial fluid pressure (IFP), and flow-induced shear stress. Mechanical changes in the environment may promote tumor progression by activating oncogenic signaling pathways or by preventing therapeutics from reaching the target site.

**Solid stress.**—Solid stress is mechanical stress that arises from the solid components in tissue (as opposed to stresses arising from fluid pressures and flows), and changes in solid stress are due to changes in both the cellular and non-cellular components of the tumor. Cell-cell and cell-extracellular matrix (ECM) dynamics, in addition to the rapid accumulation of

mass due to cell proliferation and cell recruitment, induce deformation of local tissue and can increase tumor solid stress in a process termed the ‘mass effect’ (20, 21). Furthermore, tumor ECM, including stiff collagen fibrils and incompressible hyaluronic acid, can resist tumor growth, promote apoptosis in mechanically compressed cells (22–24), and contribute to solid stress propagation in tumors (25). While these effects may impede tumor growth, studies using single cells or 3D spheroids in confined spaces or under mechanically applied compression have revealed that increased compressive stress leads to increased invasiveness of cancer cells (26–30).

Tumors are inherently biphasic, consisting of a solid phase hydrated with interstitial fluid. Thus, compressive stresses, due, for example, to a tumor growing in a confined space, result in increased fluid and solid stress, but isolating the specific contributions of solid stress in a tumor is challenging. Nia and colleagues developed methods for isolating and measuring solid stress, specifically, by quantifying the deformation of resected tumors after dissection to release residual stresses (31). When tumors bearing large solid stresses are cut along a plane, solid stresses at the newly exposed surface are released and the tumor tissue deforms in a manner dependent on local stresses prior to the cut. Using this method, the authors measured solid stress in a xenograft brain tumor as 0.21 kPa and in a xenograft pancreatic ductal adenocarcinoma tumor as 7 kPa. In another recent study by Nieskoski and colleagues, a Millar pressure sensor and wick-in-needle technique were used in concert to measure total stress and IFP locally within a tumor (32). The solid stress was then defined as the total stress less the IFP, and a solid stress of 2.67 kPa was measured in a human pancreatic adenocarcinoma xenograft. Other studies have sought to study how solid stress is spatially distributed within tumors, revealing that while the core of the tumor is often under compressive solid stress, the periphery is often under tension, and this distribution of stress is supported by computational modeling. (20, 33–35).

Matrix stiffness signals to tumor cells independent of its contributions to solid stress, and the effects of matrix stiffness on tumor biology have been well-studied using 2D and 3D models. As noted above, cells are exquisitely sensitive to mechanical signals from the extracellular environment, and in cancer cells, the responses to matrix stiffness can have downstream impacts on migration, proliferation, survival and invasiveness (23, 36–43). Moreover, gradients in stiffness and the alignment of collagen fibers may influence directional cell migration by durotaxis and alignotaxis, respectively (44).

However, while compressive stress and a rigid extracellular environment can promote invasive behavior, it is important to recognize that stiffness of the ECM is intertwined with its biochemical composition, which in itself regulates growth and invasive behavior. For example, ECM (rich in collagen I) promotes invasive behavior in 3D tumor models, as compared to laminin-rich ECM, which inhibits invasiveness (45) (36). For these reasons, there have been a number of efforts to design experimental models which enable tuning of mechanical properties independently from matrix biochemistry (46–49). Collectively, the literature points to the complex interplay between matrix mechanical properties and biochemical composition, to both promote and constrain tumor invasiveness and proliferation, depending on the context. Outside of mechanical cues that activate invasive behavior initially, the degree of motility acquired is further regulated by both ECM

composition and cellular expression of matrix remodeling enzymes, such as matrix metalloproteinases (MMPs) (Figure 3) (50).

**Interstitial fluid pressure.**—Interstitial fluid, i.e. the fluid in the spaces between cells, is estimated to comprise 20% of the body's mass and is important for oxygen, nutrients, and waste exchange between the vascular system and cells (51). The IFP of a normal tissue is close to zero and there is a small negative pressure gradient between the interstitium and capillaries, resulting in an outward transcapillary flow. This small fluid flow from capillaries to the interstitium and on to lymphatics is mainly regulated by the hydrostatic and colloid osmotic pressures of capillaries and the interstitial space (52).

In pathological conditions such as cancer, interstitial fluid that infiltrates tumor tissue from leaky blood vessels and inflammation increases IFP (53). Additionally, inadequate or non-functional tumor lymphatic vessels (e.g. due to compressive stress) can lead to insufficient drainage, which, together with altered tumor transport environment, leads to a net pressure gradient between the tumor and surrounding tissue. Elevated IFP is observed in many solid tumors (34, 52, 54). For example, Curti and colleague measured the mean IFP of melanoma nodules from 22 patients as 29.8 mmHg (55). Another study by Nathanson and colleagues measured the mean tumor IFP from ductal carcinoma patients as 29 mmHg (56). In another pancreatic ductal adenocarcinoma xenograft study, Provenzano and colleagues showed that the tumor IFP ranged from 75–130 mmHg (57). An important implication of increased tumor IFP is potentially increased difficulty in delivering therapeutics. Additionally, Hofmann and colleagues showed that the stretching of cancer cells at the tumor cortex due to IFP triggers MAPK-associated cell proliferation (58). Increased fluid pressure in tumors may cause tumor progression and metastasis due to the leaking of growth factors and cancer cells from blood vessels that are located in the tumor boundary to the surrounding host tissue (21). Furthermore, the pressure gradient in the tumor margin, because of high tumor IFP, causes higher interstitial flow into the tumor stroma and the surrounding lymphatic vessels (59). The effects of altered transport caused by elevated interstitial flow on cancer cells have been studied extensively by using different microfluidic tools. Increased interstitial flow contributes to tumor progression by promoting the activation of fibroblasts (54, 58, 60). Shields and colleagues showed that interstitial flow causes autocrine secretion of CCR-7 ligands, and therefore CCR7-dependent migration along the flow direction in three breast cancer cell lines (61). The results of a relevant study from Polacheck and colleagues supported the same idea using another breast cancer cell line: MDA-MB 231. However, they revealed that there is another CCR7-independent, integrin-activated mechanism that derives migration in the opposite side of interstitial flow (62). Another study by Polacheck and colleagues showed that the same cancer cell line reacts to interstitial flow drag by reorganizing their focal adhesions. This adaptation balances the stress at the upstream side and causes migration on the upstream direction of interstitial flow (17). Macrophages are another cell type that migrates against the flow. Li and colleagues showed that interstitial fluid flow from the tumor to the surrounding tissue not only induces M2 polarization of macrophages, but also changes their migration direction towards the tumor masses, causing M2 macrophages to contribute more to tumor progression (63).

**Flow-induced shear stress.**—Tumors in intestines, stomach, or other parts in the peritoneal cavity are exposed to movements of body fluids, such as gut contents, gastric juice, and ascites (64). In contrast to the stress that is initiated by solid components and interstitial pressure, the stresses initiated by fluid flow over tissue and cells are shear stress and drag (19). Avvisato and colleagues, who were focusing on flow-induced shear stress on colon cancer cells, demonstrated that shear stress has contradictory effects on the proliferation rate of different colon cancer cell lines (64). Fluid ascites in the peritoneal cavity are normally cleared out by the host system, however in pathological conditions such as cancer, it may build up (65, 66). Accumulation of malignant ascites is accepted as a poor clinical outcome and one of the reasons for metastasis of ovarian cancer (65). To investigate the effects of fluidic forces in a 3D ovarian cancer model, Rizvi and colleagues used a microfluidics platform (66). Results of the study have shown that 3D ovarian cancer micronodules, grown under continuous laminar flow, exhibited an aggressive and invasive phenotype, including altered tumor morphology, upregulated EGFR expression/activation, and increased EMT.

### The tumor microenvironment

A solid tumor is not only made up of genetically disrupted proliferating cells, but also a dense and rich tumor stroma containing cellular and non-cellular components of healthy tissue, such as fibroblasts, immune cells, adipocytes, proteoglycans, hyaluronan and fibrous proteins (44). The dynamic milieu where complex heterotypic interactions between cancer cells and stroma take place is called the TME (67). Abnormal angiogenesis and activation of fibroblasts are key phases during tumor progression (68). Activated fibroblasts mediate the formation of a dense, collagenous ECM in solid tumors, which is called desmoplasia (69). Furthermore, there are chemical and physical gradients in tumor tissue, such as regions of hypoxia, differences in pH, and other chemical gradients. Desmoplasia, hypoxia, and other stromal components may synergize with tumor progression and mitigate treatment success (44).

**Stromal components.**—The stroma provides structure to the tissue or organ that contains many cell types in order to maintain homeostasis (70). When cancer develops in tissue, cancerous cells come into close contact with the stroma, and genetic alterations in tumor cells change the stromal components, leading to an environment that contributes to cancer proliferation. During early cancer development, the basement membrane degrades, allowing activated stroma to come into direct contact with cancer cells (70), (71). Tumor stroma can consist of fibroblasts, immune cells, ECM components, and other tissue-specific cells, such as adipocytes. Additionally, endothelial cells and pericytes form the tumor vasculature. The accumulation of endothelial cells, fibroblasts, and immune cells within a tumor is governed by growth factors that are released by cancer and cancer-associated cells. The interactions between tumor cells and stromal cells, via soluble factors or cell-cell interactions, may cause functional changes that support tumor growth (67, 72). For example, tumor endothelial cells can become more proliferative, and quiescent fibroblasts can become activated. Another contribution of tumor endothelial cells and cancer-associated fibroblasts (CAFs) to tumor survival is mutual energy production. The metabolic heterogeneity between stromal cells and cancer cells supports the survival of cancer cells by buffering and recycling the products

of their anaerobic metabolism (73). Tumor associated macrophages (TAMs) are another type of stromal cell that is often present in the TME. Macrophages are a first line of defense against pathogens. Classically activated macrophages, which are also known as M1-polarized macrophages, can kill cancer cells. However, one of the two subgroups of macrophages, M2-polarized (a.k.a. alternatively activated macrophages) are strongly associated with cancer progression (74). Research has shown that M2 macrophages contribute to tumor progression and suppression of antitumor immunity by expressing cytokines and chemokines (75).

CAFs are considered distinct from fibroblasts that are found in normal tissue, but the sources of CAFs remains unclear (76). According to some studies, CAFs are differentiated from endothelial cells by endothelial-mesenchymal transition (68). Other theories suggest that normal fibroblasts, which are quiescent single cells located in the interstitial space, are activated by certain growth factors, such as EGF and transforming growth factor- $\beta$  (TGF- $\beta$ ), a cytokine that also plays important role in tumor progression and EMT (77, 78). An activated fibroblast is characterized by the expression of alpha-smooth muscle actin ( $\alpha$ -SMA) and shows more migratory capacity (67). Furthermore, CAFs secrete increased levels of chemokines and growth factors, which contribute to tumor progression and therapeutic resistance (79). Research has shown that CAFs play an important role in TGF- $\beta$  signaling, which regulates epithelial-mesenchymal transition (78, 80, 81). Additionally, matrix metalloproteinase-3 (MMP-3), a product of CAFs, promotes EMT by initiating the cleavage of E-cadherin. CAFs also affect dendritic cell function and inhibit cytotoxic T cell recruitment and survival that is mediated by TGF- $\beta$  secretion (82). The CAF- derived release of vascular endothelial growth factor (VEGF) also affects dendritic cell generation and maturation by reducing MHC class II expression and the ability to internalize antigens (83, 84). Lastly, CAFs can remodel the ECM by secreting ECM-degrading proteases, such as MMPs (67).

The ECM is a porous and biphasic material that surrounds the space between cells. By forming fibrils and sheet-like structures, the ECM provides structural integrity and mechanical support to the tissue (51), (85). The mechanical properties of the ECM, in part, determine the mechanical properties of the tissue (17). The main constituents of the ECM are water, proteoglycans and fibrous proteins such as collagen and elastin (86). The hypoxic and heterogeneous TME with increased inflammation results in remodeling of the ECM. This process, where inflammation occurs and the density and stiffness of ECM increases, is similar to wound healing, which is why cancer is often described as a wound that does not heal (87, 88). Additionally, the contribution of activated fibroblasts is similar in the wound-healing process and cancer, leading to a buildup of ECM components, such as hyaluronic acid and collagen (67, 89). The increase in density and stiffness of the ECM in cancer plays a vital role in tumor progression. Critical biomechanical and biochemical cues that regulate adhesion, migration, differentiation, and proliferation are, in part, provided by the tumor ECM (90, 91). Furthermore, collagen fibrils of tumor ECM may align in a way that influences metastasis or the form of migration tracks (86).

In considering the influence of ECM components on interactions in the TME, it is important to consider not only the composition of the ECM itself but also the mechanisms by which

cells interact with the ECM. For example, integrins are a family of proteins that form transmembrane heterodimers, and play a vital role in transducing mechanical signals from ECM components such as fibronectin, collagen, laminin, and vitronectin by providing a linkage to the internal cytoskeleton (92). Given the complex role of integrin-mediated signaling in regulating tumor growth and progression, it is not surprising that some integrins have emerged as therapeutic targets in cancer (93, 94). For example, fibronectin (FN) is assembled by CAFs and triggers cancer cell invasion, mostly via integrin  $\alpha_v\beta_3$  (95). Integrin  $\alpha_v\beta_3$ , which is considered a marker for breast, lung, and pancreatic carcinomas, is also associated with drug resistance (96).  $\alpha_5\beta_1$  is another integrin that has been reported to have a pro-migratory effects resulting from FN alignment by CAFs (97). In this review, we will highlight studies that investigate the impact of PDT on the ECM as well as integrin heterodimers that mediate mechanical interaction with ECM components and may form the basis for targeting strategies.

**Hypoxia and acidosis.**—Hypoxia is common in most solid tumors and is a negative prognostic indicator because of its contribution to angiogenesis, chemoresistance, and metastasis (98). As reviewed by Muz and colleagues, the oxygen concentration in tumors can decrease up to 96%, relative to comparable normal tissue (99). For example, oxygen percentage has been shown to drop from 8.5% to 1.5% in normal breast tissue versus cancerous breast tissue. Similarly, median oxygen percentages are 7.5% and 0.3% in normal pancreas tissue and pancreatic tumor, respectively. One of the main causes of tumor hypoxia is the rapid proliferation of cancer cells, which increases both the rate of oxygen consumption and the distance between blood vessels and cells (100, 101). As discussed elsewhere in this review, solid stress also causes increased hypoxia due to its compressive effects on the blood and lymphatic vessels inside a tumor. Narrowing of blood and lymph vessels leads to decreased oxygen and nutrient levels in tumor tissue, resulting in hypoxia and a decrease in pH in the TME (21, 22, 31). Furthermore, this hinders the delivery of therapeutics and facilitates the escape of cancer cells from the site of the primary tumor by increasing IFP (19). Multiple intracellular signaling pathways are triggered by hypoxia. The family of translational regulators, HIF (hypoxia-inducible factor), mediates the adaptation of cells to the hypoxic microenvironment. The accumulation and activation of HIF results in the transcriptional activation of certain genes that are involved in cell survival, angiogenesis, and invasion (102). HIF-1 also mediates a metabolic shift in cancer cells, whereby cancer cells switch from oxidative phosphorylation (aerobic) to glycolysis (anaerobic). The byproducts of anaerobic metabolism cause acidosis in the TME. Furthermore, this harsh hypoxic and acidic microenvironment creates a survival advantage for aggressive cancer cells (21). Other intracellular signaling pathways stimulated by hypoxia are PI3K/AKT and MAPK, which are associated with cell proliferation and survival (99). Furthermore, tumor hypoxia is associated with the increased secretion of TGF- $\beta$  and VEGF, and the suppression of immune response (24). Additionally, hypoxia leads to metastasis by increasing TGF- $\beta$  and proteins that are involved in EMT (103).

**The enhanced permeability and retention effect.**—The enhanced permeability and retention (EPR) effect is a feature of the TME that is characterized by abnormally high leakage of blood plasma components from the tumor vasculature, as well as their increased

retention in tumors (104). Enhanced vessel wall permeability arises from the unique physiology of the tumor vasculature. Normal vasculature in healthy tissues is hierarchically organized, enabling the efficient delivery of oxygen and nutrients (105). Tumor vasculature, on the other hand, is disorganized, tortuous, and hyperpermeable. The development of this vasculature occurs through aggressive cancer cell growth and overexpression of pro-angiogenic factors to accommodate their high metabolic needs (53, 104, 105). Tumor vessels are also immature, lacking a normal basement membrane, smooth muscle cells, and pericytes. Immature vessels, in combination with the presence of large fenestrations, cause hyperpermeability of the tumor vasculature to macromolecules and nanoparticles (52, 105–107). This enhanced extravasation is accompanied by poor lymphatic clearance, leading to the enhanced retention of extravasated particles (104, 106). The vasculature in most nonpathological tissues is less permeable than tumor vasculature, attenuating drug accumulation in healthy tissues (108). Therefore, the hyperpermeability of tumor vasculature and insufficient lymphatic drainage in tumors, in contrast to normal tissues, drive the EPR effect, which has been exploited for the selective delivery of anticancer drugs to tumor tissues (107).

The EPR effect is closely related to high IFP in tumors. The immature, hyperpermeable vasculature allows for extravasation of plasma fluid and proteins into the interstitial space, and the lack of lymphatic drainage prevents clearance, causing the fluid to accumulate (108, 109). Additionally, as the tumor grows, it compresses lymphatic vessels, further decreasing drainage of interstitial fluid (108, 110). As a result, while the IFP in normal tissue is around zero or slightly negative, tumor IFP is significantly higher and can reach above 90 mmHg (111). High IFP at the tumor site hinders the EPR effect through decreasing the pressure gradient in the interstitial space, thereby limiting the convective flow that is normally the dominant mechanism of transport in tissue for macromolecules and nanoparticles (110, 112). Therefore, a consequence of high IFP is heterogeneity in the extent of the EPR effect, limiting nanoparticle drug delivery and causing differences in therapeutic outcomes between patients. This relationship was confirmed in a mathematical model developed by Jaffray *et al.* (113) that associated liposome accumulation to the transport properties of solid tumors. They found that variations in peak IFP can cause inter-patient heterogeneity in liposome accumulation. To address the influence of abnormal vasculature on tumor IFP, anti-angiogenic therapies have been developed to normalize tumor vasculature (114). According to a model by Jain *et al.* (115), such antiangiogenic therapies can modulate the TME through increasing intratumoral convection and decreasing fluid convection into the peritumor tissue or fluid. Increasing intratumor convection improves drug penetration and distribution, while decreasing fluid convection out of the tumor decreases the likelihood of lymphatic metastasis, peritumor edema, and ascites formation.

## TARGETING THE TUMOR MICROENVIRONMENT WITH PHOTOCHEMISTRY

There is a breadth of research investigating how chemotherapeutics, biological agents, and radiotherapy can be leveraged to target the TME through preventing neovascularization, overcoming hypoxia and inhibiting the contribution of stromal cells to tumor progression (101, 116, 117). PDT is a minimally-invasive treatment modality that is used for oncologic and non-oncologic applications, and has been explored for the modulation of the TME (118–

120). The cytotoxic mechanism of PDT is based on inducing photodamage through the excitation of a non-toxic photoactive chemical, a PS, using light of a specific wavelength (121). A PS, in singlet ground state, absorbs a visible photon of appropriate energy, and becomes electronically excited to a singlet excited state. The PS then either decays to the singlet ground state by generating fluorescence or undergoes intersystem crossing to the triplet excited state. From there, the molecule can interact directly with molecular oxygen or with another substrate via electron or proton transfer to return to the singlet ground state. When these photochemical reactions result in the generation of sufficient amounts of singlet oxygen or other reactive molecular species to cross the threshold of cell survival, toxicity is conferred to the target tissue, such as cancer cells (121, 122).

The modes of cell death activated by PDT (e.g. apoptosis, necrosis, necroptosis, lethal autophagy, or paraptosis) depend strongly on the type of PS used and the sub-cellular and/or extracellular PS localization at the time of light activation. Mitochondrial photodamage generally leads to the initiation of apoptosis through the direct release of cytochrome c and degradation of the anti-apoptotic Bcl family of proteins, without effects on pro-apoptotic Bax (5, 123, 124). The initiation of apoptosis by direct mitochondrial photodamage is significant in cancer therapy because this pathway bypasses many of the escape and repair mechanisms through which cancer cells are able to develop resistance to traditional treatments. PDT can also cause toxicity by initiating necrosis, and causing white blood cells to release cytokines, such as necrosis factors (125, 126). Depending on the type of photodamage and the underlying biology of the target cells, PDT can also cause lethal levels of autophagy (127), or as has been reported more recently, can trigger paraptosis, a response, in part, to misfolded ER proteins (128). Combining PS or PS formulations to enhance PDT- efficacy by targeting complementary tumor compartments or sub-cellular sites, has also shown promise (129–136). Importantly, because the timing, location, and intensity of light activation can be carefully controlled, PDT is inherently a targeted therapeutic modality. Exploitation of PDT-mediated cytotoxic mechanisms has been used in combination with conventional and emerging therapies to overcome chemoresistance (137) and to enhance efficacy (138–143).

In addition to inducing spatially and temporally selective cytotoxicity, PDT can also alter the TME by targeting specific malignant and non-malignant cells as well as extracellular components. This damage is highly dependent on PS localization, which is dictated by the structural and chemical properties of the PS and the drug light interval (the time interval between drug administration and light exposure). Some PSs localize preferentially in specific tumor compartments, such as Tookad, which largely stays in the vasculature (144), (145). Other PSs, such as benzoporphyrin derivative (BPD), can target various tumor compartments (e.g., vasculature, ECM, cancer cells) depending, in part, on the PS-light interval. The use of Tookad and BPD in the context of vascular-targeted PDT is further discussed in a later section. The localization of the PS directly influences the treatment outcome. For PDT targeted to the vasculature, there can be a range of effects, including vascular permeabilization for the enhanced delivery of nanoscale therapeutics, as well as blood vessel occlusion which can result in increased tumor hypoxia (146–149). Additionally, PDT that is targeted to the ECM can alter ECM composition through hyaluronic acid degradation and collagen crosslinking (150–152). PDT-induced collagen crosslinking is

being researched for the treatment of Keratoconus of the cornea and intimal hyperplasia mediated vascular cell migration (153, 154). However, PDT can also lead to the degradation of collagen through damaging fibroblasts and causing the release of MMPs (155), (156). While the degradation of collagen prevents desmoplasia and increases drug penetration, it may also lead to increased invasion and metastasis of cancer cells (Figure 4) (91, 157), (158). Therefore, the effects of PDT on ECM composition can be highly varied, and destruction of stromal components does not necessarily improve treatment outcomes. For example, previous work has shown that the depletion of the heterogeneous stroma results in more aggressive cancer behavior. In one study, a transgenic mouse was generated with the ability to delete  $\alpha$ SMA<sup>+</sup> myofibroblasts in pancreatic cancer, and the depletion of the stroma caused invasive tumors with diminishing mouse survival (159). Therefore, destruction of stromal components could aggravate disease progression, so PDT treatments designed to target stromal components must be carefully studied and optimized. A summary of PSs that are commonly used in clinics and in preclinical research is presented in Table 1, along with their localization characteristics (132, 160–170).

Spatiotemporal targeting is inherent to PDT, based on PS localization properties, PS-light interval and spatial confinement of light-based activation. Targeted PDT can also be achieved by conditioning the target cells prior to PDT in order to improve the intracellular accumulation of the PS. This strategy has primarily been implemented for the targeting of PpIX. The specific delivery of PSs to particular tissues and cell types can also occur through incorporating monoclonal antibodies, nanoparticles, and targeting moieties into PDT treatment strategies. This review categorizes approaches for achieving targeted PDT into three central areas: I.) targeting by cellular and tissue modulation, II.) functional targeting, and III.) targeted delivery. Targeting by cellular and tissue modulation refers to methods that strategically alter biological processes to improve PDT treatment efficacy. Functional targeting encompasses approaches that leverage the unique properties of the TME, such as abnormal vasculature as well as tumor hypoxia and acidity, to achieve selectivity. The third strategy, targeted delivery, involves approaches that use drug and PS carriers, and targeting moieties, for selective tumor destruction. This chapter discusses these various methods for targeting PDT to different components of the TME, as well as the effects of photochemistry on the mechanical and stromal interactions in tumors.

### Targeting by Cellular and Tissue Modulation

Cellular and tissue modulation is among the most translationally-relevant approaches to enhance selective treatment of target tissue. In the case of targeted PDT for cancer, a major goal of this targeting strategy is to increase the accumulation of PSs in tumors. Cellular and tissue modulation, also known as biomodulation, is the alteration of biological mechanisms by which cells metabolize, take up, or retain PSs to selectively enhance PDT efficacy (12). A focus of this section of the review is the heme biosynthesis pathway utilized in mammalian cells, also known as the Shemin pathway, which involves the conversion of 5-ALA to heme (171–177). The penultimate step in this pathway is the production of PpIX (Figure 5a). A preferential site of localization of PpIX is the mitochondria (169). PpIX can also be transported into the cytosol by mitochondrial ATP-binding cassette transporter G2 (ABCG2) (178, 179). The production of PpIX can be increased by modulating key enzymes in the

heme pathway, such as coproporphyrinogen (CPO) and ferrochelatase (FECH), as summarized in Table 2. ALA is a prodrug that is approved by the FDA for fluorescence-guided resection of gliomas and photodynamic treatment of actinic keratosis (180–183).

Preferential accumulation of PpIX in cancer cells results from dysfunctional activity of key enzymes in the heme pathway compared to healthy tissue. This includes decreased FECH activity and increased CPO activity, which increases the production of PpIX and limits its conversion to heme, leading to increased accumulation of the PS in target tissue (184). In 1979, Malik and Djaldetti first showed that ALA enhanced the synthesis of hemoglobin as well as the intermediate precursor uroporphyrin and PpIX (185). Later, in 1987, eighty basal cell carcinomas (BCC) were treated using ALA-induced PpIX-based PDT by Kennedy and Pottier, with a 90% response rate (186–188). Among the advantages of PpIX is that it is rapidly cleared from the skin from topical, systemic, oral, or intradermal administration, thereby minimizing skin phototoxicity (189, 190). Difficulties arise in using this treatment due to insufficient or non-uniform PpIX accumulation in the cancerous tissue, highlighting the need for approaches such as cellular and tissue modulation.

To overcome the problem of insufficient or non-uniform PpIX accumulation, various methods to modulate the heme pathway have been developed. Cells that are poorly differentiated and rapidly proliferating, as is common in many tumors, may produce insufficient amounts of PpIX (12). One area of investigation to address this issue involves the use of cellular differentiators such as vitamin D or its analogues, methotrexate, retinoic acid, 5-fluorouracil acid, iron chelators, and the androgens 5 $\alpha$ -dihydrotestosterone (DHT) and R1881 (191–217).

Treatment efficacy can be influenced by microenvironmental factors such as temperature, pH, and oxygen availability/hypoxia (218–223). An emerging area of investigation is the role of mechanical stress on PpIX production and accumulation. A study by Niu *et al.* (13) using two glioma cell lines of bulk/solid tumors, U373 and U118, showed that cell proliferation rate increased in a nonlinear fashion as substrate stiffness increased. Their motivation was to study cell proliferation and PpIX synthesis in glioma cells on substrates simulated the tissue stiffnesses of normal brain (1 kPa) and glioblastoma (12kPa) (13, 224, 225). The two cells used to evaluate the effects of mechanical stiffness were selected based on a previous study of PpIX production in a panel of 10 glioma cell lines. U373 was determined to produce the most PpIX, while U118 produced the least (226). Evaluation of PpIX production in these two cell lines on substrates of varying stiffnesses showed that the mechanical properties of the underlying matrix affected each cell line differently. In the U118 cell line, a stiffer microenvironment led to increased PpIX accumulation with no change in the rate of cell growth (13). In contrast, substrate stiffness had no significant effect on PpIX accumulation in the U373 cell line (Figure 5b) (13). In another study by Ulrich *et al.*, micromechanical cues from the ECM on glioma cells was evaluated by testing various ECM substrates with different mechanical and biochemical properties. It was found that ECM elasticity strongly affects glioma cell structure, motility, and proliferation (224). These findings highlight the impact of the ECM on cell morphology, cytoskeletal organization, motility, and control of MMII-mediated intracellular contractility (224). Further studies must

be done to clarify the effects of mechanical stiffness on PpIX production in glioma and other cell types.

### Functional targeting

The TME acquires a unique set of biochemical traits which are closely connected to tumorigenesis (227). While this distinct TME is linked to proliferation and metastasis, it also provides opportunities for the development of PSs that target these unique features through a strategy termed *functional targeting*. One aspect of functional targeting is the EPR effect, which causes an increase in nanoparticle accumulation and retention at the site of a tumor due to hyperpermeable tumor vasculature and insufficient lymphatic drainage. This phenomenon has been exploited for cancer treatment through the design of nanomedicines for drug targeting to tumors, though there is significant heterogeneity that limits the influence of the EPR effect on drug localization (109, 228, 229). Various photochemistry-based strategies have been employed to enhance the EPR effect through vascular permeabilization to improve nanomedicine extravasation in tumors. PDT has also been used clinically to occlude vasculature, with the goal of restricting the supply of blood to the tumor. Other unique features of the TME that are leveraged to achieve *functional targeting* include hypoxic and acidic conditions (Figure 6). As discussed in a previous section, tumors develop hypoxic conditions due to an imbalance in oxygen supply and consumption (100). The abnormal, disorganized vascular network that develops in the TME provides limited oxygen supply despite an increasing demand for oxygen generated by the rapidly growing tumor. One of the byproducts of the hypoxic microenvironment is the development of acidic conditions (230). In the absence of oxygen, cancer cells use anaerobic glycolysis, leading to lactic acid production and a decrease in pH (231). Interestingly, even when oxygen is abundant and mitochondria are fully functional, cancer cells can utilize aerobic glycolysis, producing lactate despite the availability of oxygen. This is termed the Warburg Effect, first described in the 1920s by Otto Warburg (232). This section will discuss functional targeting, through which the unique features of the TME, such as abnormal vasculature, acidity, and hypoxia, can be leveraged to achieve targeted PDT.

**Exploiting the enhanced permeability and retention effect and targeting the tumor vasculature.**—The EPR effect, first discovered by Matsumura *et al.* (233), allows nanoscale drugs to passively accumulate preferentially at the site of a tumor. While many nanomedicine formulations claim to leverage the EPR effect for specific delivery to cancer cells, there has been recent discussion regarding critical limitations to the EPR effect and the models used to study it that hamper its true influence on nanoparticle accumulation (234). PDT has demonstrated potential as a strategy to address limitations in the EPR effect and increase nanoparticle extravasation at the tumor site.

As discussed in a previous section, the EPR effect occurs due to the tendency of tumors to develop abnormal vascular networks with poor lymphatic drainage. An imbalance of pro-angiogenic and anti-angiogenic signals at the tumor site leads to the development of abnormal vascular networks, which often lack the organization of typical vascular networks, and are tortuous and dilated (53). This vascular network often exhibits large pores, enabling increased extravasation of nanoparticle drug carriers into the tumor site for heightened drug

accumulation. Vasculature in nonmalignant tissue has smaller fenestrations, thereby limiting drug accumulation in healthy tissues and minimizing adverse side effects (108). The high extravasation in tumor vasculature is accompanied by poor lymphatic drainage, leading to increased drug retention (235).

Knowledge of the EPR effect has had widespread effects on the development of drug delivery systems for cancer, and various PS delivery vehicles have been developed that leverage the EPR effect to increase intra-tumoral PS accumulation (236). Examples of nanoparticle delivery vehicles that have been employed for PS delivery include liposomes (237–241), quantum dots (242, 243), viruses (244, 245), virus-like particles (VLPs) (246, 247), polymeric nanoparticles (248), silica nanoparticles (249, 250), metal nanoparticles (251), carbon nanoparticles (252, 253), and others (254–256). While the EPR effect has been used as the foundation for the development of a myriad of nanoparticle drug delivery systems, it has shown limitations in clinical practice due to high heterogeneity (109). Due to this heterogeneity, predictive nanotechnology is being developed to determine if a tumor is likely to accumulate nanoparticle drugs through EPR (257). For example, ferumoxytol (FMX), a 30 nm magnetic nanoparticle, when administered intravenously and visualized with magnetic resonance imaging (MRI), was shown to predict both the intratumoral accumulation of therapeutic nanoparticles, as well as their anti-tumor effect (258, 259). Such technology could be used to stratify patients based on EPR effect, and therefore has significant implications for personalized medicine in oncology.

The heterogeneity of the EPR effect occurs due to a variety of parameters that can differ widely between tumors, such as vascular maturation, tumor cell density, type of cancer, and IFP, among many others (109, 260, 261). Additionally, there are key limitations in how the EPR effect is studied. For example, it has primarily been evaluated in implanted tumors rather than metastatic tumors, despite the fact that metastases account for 90% of cancer deaths (234, 262). Additionally, the EPR effect is primarily studied in animal models that poorly recapitulate human tumors (263). Due to heterogeneity in the extent of the EPR effect, methods to improve permeability of tumor vasculature are of great interest for improving drug delivery and therapeutic outcomes. PDT has been utilized as a novel method to achieve this. For example, through understanding pharmacokinetics following PS administration, drug-light interval can be optimized to activate PSs while they are accumulated in specific compartments to increase permeability to macromolecules and nanoparticles (8, 140, 143, 264, 265). Moreover, targeting moieties can be utilized to achieve accumulation of PSs in the tumor vasculature and perivascular tumor cells to enhance the EPR effect (266–268).

A study by Snyder *et al.* (147) explored the ability of low-irradiance PDT to permeabilize vessels to macromolecular agents, including fluorescent microspheres and liposomal doxorubicin (Doxil). Using a murine colon cancer model, when fluorescent microspheres were administered immediately following PDT, there was an increase in intratumoral accumulation of microspheres in the range of 0.1 to 2  $\mu\text{m}$ . When using low fluence rate PDT prior to Doxil administration, there was a significant increase in Doxil content in tumors, as well as up to 80% long-term tumor control. Additionally, a study by Gil *et al.* (148) used PDT as a method to improve the delivery and efficacy of oncolytic vaccinia virus (OVV) to

primary and metastatic tumors in mice. Tumors that received PDT treatment ( $128 \text{ J cm}^{-2}$  at  $14 \text{ mW cm}^{-2}$ ) 12 hours prior to OVV administration had an over 10-fold increase in viral titres compared to the control tumors. However, PDT with other light doses ( $135 \text{ J cm}^{-2}$  at  $75 \text{ mW cm}^{-2}$  and  $48 \text{ J cm}^{-2}$  at  $7 \text{ mW cm}^{-2}$ ) was less effective, underscoring the importance of treatment optimization when using PDT to increase intratumoral accumulation of therapeutics.

While PDT alone can be used to permeabilize tumor vasculature, targeting moieties can be incorporated to increase binding affinity to target cells. For example, Zhen *et al.* (267) synthesized PS-encapsulated ferritin nanocages with peptides targeted to  $\alpha v \beta_3$ , an integrin that is overexpressed in neoplastic endothelial cells. When used prior to nanoparticle administration, these endothelium-targeted PSs caused an increase in nanoparticle accumulation in tumor tissues by  $\sim 20$ -fold. When targeted PDT was used prior to treatment with the anti-cancer drug Doxil in a xenograft breast cancer tumor model, an increase in treatment efficacy of 75.3% was observed. SEM images confirmed that the increased nanoparticle accumulation could be attributed to large fenestrae in the endothelial walls. Additionally, Sano *et al.* (268) showed that photoimmunotherapy (PIT) can be used to achieve an increase in the EPR effect, termed the super-enhanced permeability and retention (SUPR) effect. Following the light activation of panitumumab-IR700 constructs that accumulated in perivascular tumor cells, they observed significantly higher extravasation of a variety of nanoscale particles. This is attributed to the ability of PIT to cause necrosis specifically in the antigen-expressing cancer cells in the perivascular space while leaving nearby normal cells unharmed (269).

Previous work has also demonstrated that PDT can cause time-dependent changes in tumor IFP, which is normally a barrier to EPR-mediated drug accumulation in tumors (110, 112, 270). A study by Leunig *et al.* (270) showed that PDT caused an initial increase in tumor IFP immediately following light administration, but 24 hours after PDT, tumor IFP dropped to 50% of the control in a hamster melanoma model. In another study, Perentes *et al.* (149) investigated the IFP alterations caused by PDT as well as the resulting changes in the accumulation of liposomal doxorubicin. The authors showed that low-dose photodynamic therapy (L-PDT) reduces tumor IFP without impacting normal tissue IFP. Additionally, when liposomal doxorubicin was administered following L-PDT treatment, the drug penetrated significantly deeper into the tumor tissue compared to the control. Recently, Cavin *et al.* (271) explored the mechanism through which L-PDT alters vasculature to modulate IFP. Using a pericyte and endothelial cell coculture, they found that PDT alters the function of pericytes, strengthening their association with endothelial cells. Tumor vasculature typically has poor pericyte coverage (52), so increasing pericyte-endothelial cell association causes vascular normalization, leading to a decrease in IFP and an increase in convective transport of drugs into the tumor. These studies illustrate that PDT can have significant effects on tumor IFP, though the effects are dependent on many factors, including PS-light interval, light dose, and PS dose.

In addition to permeabilizing the vasculature to improve drug accumulation, vascular-targeted PDT has also been used to cut off blood supply to the tumor (272–275). WST-09 (Tookad, palladium bacteriopheophorbide) and WST-11 (Tookad soluble, Padeliporfin) are

PS with limited extravasation from vasculature, enabling spatially selective photodamage by confining PDT-mediated toxicity to the vascular compartment. When activated by light, Tookad can cause vascular occlusion, resulting in tumor necrosis (276–279). Clinical trials have confirmed that PDT using Tookad is a well-tolerated and effective approach for the treatment of prostate cancer (280–282). In one clinical trial, 28% of patients who received vascular-targeted PDT experienced disease progression after 24 months, compared to 58% of patients who underwent active surveillance (281). An extended follow-up study analyzed the same cohort and found that, at four years, patients who had received vascular PDT experienced significantly lower disease progression rates and were less likely to convert to radiotherapy (24% versus 53%) (282).

A primary, nononcologic, application of PDT for vascular occlusion is in the treatment of age-related macular degeneration (AMD), the leading cause of blindness in elderly adults in western countries (283, 284). The neovascular form of AMD (also known as exudative or “wet”) leads to the formation of aberrant blood vessels from the choriocapillaris under the foveal avascular zone below the retina (subfoveal choroidal neovascularization, CNV). These leaky blood vessels can cause loss of vision through subretinal hemorrhage and detachment of the retinal pigment epithelium (283). Although thermal photocoagulation has been used as a primary treatment method for destroying CNVs, this treatment method has drawbacks, including retinal scar formation and immediate loss of visual acuity (284, 285). Preclinical and clinical studies demonstrated that CNV occlusion and improvements in visual acuity were achieved by activating intravenously administered BPD with non-thermal laser irradiation at short PS-light intervals (while the PS is in the vasculature) (161, 286–289) to cause thrombosis of vessels and selective damage to endothelial cells (285). Vascular-targeted PDT with a liposomal formulation of BPD, Visudyne, is approved worldwide for the treatment of wet AMD and has been used in millions of patients. PDT can therefore have multiple effects on the vasculature, including permeabilization and occlusion, depending on treatment parameters. This underscores the necessity for carefully optimizing vascular targeted PDT treatment regimens to achieve specific, desired effects.

While PDT has great potential in the clinic to induce cytotoxicity in cancer cells, it has also emerged as a promising modality to improve the extravasation of nano- and micro-scale agents at the tumor site. This has been achieved either through targeting the vasculature directly, or through targeting the cells in the perivascular space. Additionally, localization of PS in specific regions, such as vasculature or perivascular cancer cells, can be achieved through the use of targeting moieties as well as through the optimization of drug-light interval. While preclinical studies are promising, there is more work that must be done to bring this treatment strategy from benchtop to bedside. For example, light delivery methods can be further engineered to ensure that light is delivered uniformly to maximize access to target tissues. Additionally, the parameters for PDT treatment must be further optimized to improve drug delivery. For example, varying PS concentration and the amount of light delivered to the vasculature could potentially change the size and number of fenestrations created in the endothelial walls. Optimizing these parameters is critical because nanomedicines vary in size. Similarly, the optimal time after PDT to administer nano- and micro-scale therapeutics could differ based on particle size, so more work should be done to characterize this thoroughly. Moreover, combinational treatment regimens that pair

carefully optimized EPR-enhancing PDT with nanoscale therapeutics still need to be evaluated in clinical trials to determine if this strategy can successfully overcome the heterogeneous EPR that often limits nanomedicine efficacy. Overall, due to the potential of PDT as an adjuvant to nanoscale cancer drugs, the development of these technologies could have extremely widespread implications for cancer therapeutics.

**Targeting the hypoxic microenvironment.**—Another feature that can be exploited for PS targeting is the hypoxic TME. Piao *et al.* (290) developed a PS that can only be activated under hypoxic conditions, such as those in solid tumors. They conjugated an azo moiety to a seleno-rosamine dye, blocking intersystem crossing and therefore blocking singlet oxygen generation. Under low oxygenated conditions, the moiety is cleaved from the dye, allowing singlet oxygen generation upon light activation. The drug, therefore, has increased specificity for the hypoxic TME, where it can be light-activated.

Additionally, PDT can induce hypoxia in tumor tissues through both reducing vascular perfusion and consuming oxygen (146). One study by Busch *et al.* (291) investigated how two different fluence rates, 38 mW/cm<sup>2</sup> and 75 mW/cm<sup>2</sup>, differentially impacted Photofrin-PDT-mediated oxygen depletion. They found that in tumors treated with 75 mW/cm<sup>2</sup>, there was significant hypoxia, even tissues in close proximity to the blood supply (vascular-adjacent tissues). Alternatively, in tumors treated with 38 mW/cm<sup>2</sup>, there was an insignificant increase in hypoxia in vascular-adjacent tissues. The oxygen consumption associated with PDT presents an issue for repeated PDT treatments, where decreasing oxygen levels in the tissues might diminish the efficacy of subsequent treatments. While this oxygen consumption can exacerbate hypoxia-associated proliferation and metastasis, some groups have taken advantage of this by developing treatments that incorporate both PSs and hypoxia-activated prodrugs for a synergistic effect. For example, Feng *et al.* (292) developed a liposome containing chlorin-6 in the hydrophobic bilayer and AQ4N in the core. Chlorin-6 is a second-generation PS that is activated by 660 nm light, and AQ4N is a hypoxia-activated prodrug. In hypoxic conditions, nontoxic AQ4N is reduced first to AQ4M, then to AQ4, which intercalates DNA to inhibit topoisomerase II (293). They found that following irradiation of the liposome, chlorin-6 is activated, inducing severe hypoxia. These hypoxic conditions catalyzed the activation of AQ4N, leading to significantly decreased tumor volume in a mouse model.

Similarly, Liu *et al.* (294) used upconversion nanoparticles (UCNPs) to encapsulate both a PS, silicon phthalocyanine dihydroxide (SPCD), and a bioreductive prodrug, tirapazamine (TPZ). Tirapazamine generates cytotoxic free radicals, which in aerobic conditions are oxidized back into the harmless parent compound. However, under hypoxic conditions, TPZ becomes highly reactive and causes strand breaks in DNA (295). Liu *et al.* (294) found that under normal physiological oxygenation conditions, TPZ had little cytotoxicity, but following PDT-induced hypoxia, TPZ toxicity increased significantly. These studies illustrate that PSs can be functionally targeted to the hypoxic TME conditions, inducing specific toxicity.

Moreover, there are many studies that show that the therapeutic effect of PDT can improve when combined with methods for decreasing hypoxia (296, 297). Some methods focus on

increasing the oxygen concentration by using oxygen carriers such as artificial red blood cells that contain PSs (298–300). Mild heating also improves the intertumoral blood flow in PDT. The process is called photothermal therapy (PTT) in which the rapid increase in temperature above the threshold value of 42–45 degrees Celsius can kill cancer cells (301). Performing fractional PDT treatment can also minimize the dependence on oxygen by allowing time for the replenishment of tissue oxygen (302).

**Targeting the acidic microenvironment.**—The low pH of tumors has also been exploited in the development of functionally targeted PSs. For example, Luo *et al.* (303) developed a self-transformable pH-driven membrane anchoring photosensitizer (pHMAPS) for acidic selectivity. The pHMAPS incorporates pH low insertion peptide (pHLIP), which adopts a random coil configuration in neutral physiological pH (~7.4), but changes conformation in a more acidic pH to an  $\alpha$ -helix structure. This conformational change induces insertion into the cell membrane for selective delivery of the PS to cells in acidic environments such as the TME (303), (304). In an *in vivo* biodistribution analysis, pHMAPS localization was compared to a peptide without the ability to change conformation, nonmembrane anchoring photosensitizer (NMAPS). They found a significantly higher accumulation of the pHMAPS in the tumor compared to NMAPS, demonstrating successful targeting of the acidic TME.

Another study that leveraged the unique properties of pHLIP was done by Yu *et al.* (305), in which pH-responsive hollow gold nanoparticles loaded with PSs were synthesized to target the acidic microenvironment. In this study, pHLIP was conjugated to the nanoparticle surface for selective delivery to cells at low pH. Upon light activation, the nanoparticles produce heat for photothermal therapy, and release the PS, chlorin-6. Following release, chlorin-6 interacts with oxygen in surrounding tissues to generate reactive oxygen species for PDT.

Han *et al.* (306) engineered a chimeric peptide, PAPP-DMA, for targeted uptake by cells in the acidic TME. The peptide contains PpIX conjugated to a nuclear localization sequence (NLS) that has been modified with a negatively charged 2,3-dimethylmaleic anhydride (DMA). DMA detaches in acidic pH, increasing the charge of the peptide. This charge increase potentiates higher electrostatic interactions with the negatively charged cell membrane, facilitating internalization of the drug. Following cellular uptake, subcellular targeting of the nucleus is achieved through the NLS. This targeting strategy employs multiple layers of specificity to achieve cellular uptake in acidic conditions, as well as subcellular specificity to translocate the PS to the nucleus for light-activated DNA damage. Gao *et al.* (307) also leveraged a masked targeting peptide to target the acidic TME. They decorated the surface of a PS-loaded polymeric nanoparticle with TAT, a cell-penetrating peptide (308). To create specificity for low pH, the TAT peptide was modified with 2,3-dimethylmaleic anhydride to mask the cell-penetrating activity. Acidic pH reactivates the peptide's targeting activity, facilitating nanoparticle internalization by cells in the TME. Overall, these studies illustrate that biochemical interactions between the acidic TME and drug delivery vehicles can be leveraged to enable improved drug delivery to tumors.

## Targeted Delivery

While PSs are potent cytotoxic agents, for many formulations, clinical translation is limited by their hydrophobic nature. This causes the drugs to aggregate, thereby limiting ROS production and decreasing cytotoxicity (236). Conjugating PSs to nanoscale carriers can improve their solubility, facilitating effective delivery to, and uptake by, target cells. Nanotechnology has therefore advanced PDT by providing a mechanism to improve PS delivery. In addition to providing a vehicle for PS delivery, nanoparticle carriers can also be functionalized to specifically target cancer cells and cancer-associated stromal cells. This strategy, termed *targeted delivery*, refers to the use of antibodies as nanocarriers and targeting moieties, or the functionalization of nanocarriers and PSs with targeting moieties, to achieve specific PS delivery. This method of targeting leverages the expression of molecular biomarkers on target cells that are less expressed on other cells to selectively enhance PS accumulation and decrease systemic toxicity.

**Photoimmunotherapy.**—PIT is a form of targeted PDT that leverages the molecular targeting capabilities of antibodies and the selective phototoxicity of PSs to achieve cell-specific drug delivery (Figure 7a, 7b). This is achieved through the synthesis of photoimmunoconjugates (PICs), which are drug conjugates composed of antibodies and PSs. Cell targeting is typically dependent on the presence of membrane proteins that are differentially expressed on diseased versus normal tissue (309). Ideally, these membrane proteins should have lower expression, or none at all, on healthy cells in order to maximize specificity and minimize systemic toxicity. An antibody binding to a target can trigger receptor-mediated endocytosis, followed by proteolytic cleavage to degrade the conjugate and release the PS (310). Subsequent light irradiation leads to phototoxicity (Figure 7a) (309). Some of the cell surface receptors that serve as targets for PIT are also implicated in metastasis and proliferation. Depending on the dose, binding of the PIC can also inhibit activation of these pathways. Therefore, PICs can serve dual functions through receptor inhibition and selective delivery of the PS for PDT (311).

PIT was first described in 1983 in the lab of Julia Levy by Mew *et al.* (312). It was discovered that by conjugating the PS, hematoporphyrin, to monoclonal antibodies specific for myosarcoma, it was possible to more significantly inhibit cancer growth *in vivo* than treatment with either monotherapy alone, as well as treatment with the unconjugated antibody and PS, where all groups were light-activated. This study demonstrated for the first time that PICs can selectively enhance photodestruction of tumors. This discovery led to the development of many PICs incorporating a variety of antibody-PS combinations (84, 85, 313–315).

For example, in elegant work by Savellano and colleagues (316), the PIC conjugation and purification processes were optimized to produce highly pure PICs composed of the EGFR-specific antibody, C225, and the PS, BPD. Their optimization overcame previous issues with PIC development, including large amounts of impurities (unconjugated PSs), as well as the presence of insoluble aggregates. These issues presented a significant problem for earlier studies, because the therapeutic effects of the PICs cannot be clearly distinguished from the effects of the aggregates and impurities (316). In another study, Mitsunaga *et al.* (317)

synthesized PICs composed of EGFR-specific monoclonal antibodies (trastuzumab and panitumumab) and the PS, IRDye 700DX (IR700). It was shown that cytotoxicity was only conferred to EGFR-expressing target cells, demonstrating the selectivity of the conjugate. The authors hypothesized that fluorescence generated from light-activation of the PIC could be used for theranostic applications. Similarly, Spring and colleagues used the Cetuximab-BPD PIC developed by Savellano and colleagues to target EGFR expressing ovarian cancer micrometastases. In this PIC, the BPD molecules are self-quenched and not photoactivatable until they are internalized and proteolytically cleaved in target cells, further enhancing PIT selectivity and limiting off-target toxicity (318).

PIT has also been shown to trigger host anti-tumor immunity. A recent study by Ogawa et al. (319) explored a mechanistic understanding of cell death following PIT with mAb-IR700 PICs. The authors suggest that, unlike conventional PDT which is dependent on the generation of reactive oxygen species, cytotoxicity induced from PIT using near infrared light (NIR-PIT) is a result of membrane destabilization followed by water influx and cell bursting. This also elicits the maturation of immature dendritic cells, creating a concurrent immunological response to the tumor. A follow-up study was conducted to explore the cause of membrane destabilization (320). It was shown that upon activation with NIR light, axial ligands on IR700 dissociate, decreasing the hydrophilicity of the dye. The authors postulate a conformational change in PIC-antigen complexes following PIT that confers stress to the cell membrane, resulting in water influx and cell bursting.

While PIT has been primarily targeted to malignant cells, it can also be used to target cancer-associated stromal components in the TME. For example, work by Zhen *et al.* (321) demonstrates that CAFs can be targeted using PIT. CAFs are present in the tumor stroma and support tumor growth and metastasis by remodeling the ECM and secreting growth factors, cytokines, and chemokines (322). In this study, ferritin, which serves as a cage for the PS, ZnF16Pc, is coated with fibroblast-activation protein (FAP)-specific single chain variable fragment (scFv) to target FAP, a protein overexpressed on the surface of CAFs. One particular feature of CAFs that the authors were interested in studying was CAF-mediated T cell exclusion. By this mechanism, CAFs are able to prevent T cells from reaching the tumor through either the deposition of a dense ECM, or the production of CXCL12. It was found that CAF-targeted PIT led to a significant increase in T cell infiltration into the tumor, demonstrating that PIT can be used to modulate the tumor stroma and potentiate an immune response (Figure 7b). Importantly, the ECM deposited by CAFs is also known to provide a critical barrier to nanoparticle delivery to tumor cells (323). Therefore, Li *et al.* (324) investigated the effect of CAF-targeted PIT on nanoparticle delivery to 4T1 tumors. The authors found that by treating with CAF-targeted PIT two days prior to nanoparticle injection, intratumoral nanoparticle accumulation was improved in a particle size-dependent manner. While the effects of CAF-targeted PIT on stromal properties have been investigated, more work is required to evaluate the impact of these treatments on the mechanical properties of the TME, as well as the inverse.

While PIT is a powerful tool for targeted PS delivery, PICs have several limitations, including a relatively low PS-to-antibody ratio (typically 2 to 7 PSs per Ab), poor intracellular PIC accumulation, and limited target presentation on cells. However, PIT can be

interfaced with nanotechnology to overcome these limitations. We have previously shown that multiple PICs can be conjugated to nanoparticles (PIC-NP) for enhanced PIC uptake, as has been shown in ovarian cancer and glioblastoma cells (325). PIC-NP increases the intracellular accumulation of PIC in cancer cells by at least two-fold compared to PIC alone, which was attributed by the authors to the “carrier effect”. This phenomenon describes the indirect endocytosis of multiple PICs that are conjugated to the nanoparticle surface. Therefore, one binding event between an antibody and a target cell leads to the uptake of multiple antibodies, each with multiple PSs. This study suggests a promising direction for the future of PIT by providing a mechanism to overcome key limitations of PICs.

#### **Modified nanocarriers for cancer cell and stromal cell targeting.—**

Nanotechnology-assisted PS delivery to tumors relies on the passive accumulation of PS-nanoparticle conjugates due to the EPR effect, which is discussed in an earlier section of this review. However, upon accumulation at the tumor site, the PS can interact nonspecifically with cells in the TME. To achieve higher specificity for target cells and avoid damage to nearby healthy tissue, nanoparticle surfaces have been functionalized with various targeting moieties for molecular recognition. Similar to PIT, these functionalized nanoparticles rely on the high expression of molecular targets on the surface of target cells for specific delivery (Figure 8a). Targeting moieties used for this purpose include folic acid, peptides, antibodies, and carbohydrates (325–338) (Table 3), and can be either conjugated to a nanoparticle or directly to a PS.

Most PDT treatments that incorporate such targeting moieties are designed to directly target cancer cells. Selectively killing cancer cells can attenuate growth-induced solid stress, which is generated through interactions between structural components of the microenvironment, as discussed in previously (25).

By attenuating growth-induced solid stress, cancer cell targeting can modulate the mechanical properties of the TME. Additionally, as outlined previously, the noncancerous cellular components of the stroma can contribute to cancer progression and the mechanical stresses on the tumor. For example, CAFs and TAMs play key roles in tumor evolution through increasing malignant potential and chemoresistance (339). The noncancerous cells in the tumor stroma are therefore attractive targets for cancer therapies, and PS delivery vehicles have been designed with targeting moieties against non-cancerous stromal cells.

One method for cancer cell targeting is using carbohydrates as targeting moieties. Carbohydrates have a high binding affinity to lectins, which are endogenously overexpressed on the surface of cancer cells as well as non-cancerous stromal cells (340). A recent study in 2018 by García Calavia *et al.* (328) demonstrated that carbohydrates can serve dual functions as both targeting moieties and nanoparticle stabilizers. The investigators synthesized gold nanoparticles conjugated to zinc phthalocyanine, and the carbohydrate, lactose. Here, lactose conjugated to the surface was effective as a targeting moiety for the galectin-1 receptor on breast cancer cells, and also helped to stabilize the nanoparticle in aqueous solutions. This is an example of “direct lectin targeting”, where an exogenous carbohydrate is incorporated into the drug delivery system to target endogenously expressed carbohydrates. The binding affinity between carbohydrates and lectins can also be leveraged

by decorating the nanoparticle surface with lectins in order to target carbohydrates overexpressed on the surface of cancer cells. This strategy is termed “reverse lectin targeting” (341). The latter strategy was investigated in a study by Obaid *et al.* (331) that compared using lectin for carbohydrate-targeted nanoparticles with using antibodies for protein receptor-targeted nanoparticles. To study this, gold nanoparticles were functionalized with either the lectin, jacalin, or a monoclonal antibody. Both constructs used zinc phthalocyanine (C11Pc) as the PS, and efficacy of the constructs was evaluated in multiple cancer cell lines: HT-29 colon cancer cells and SK-BR-3 breast cancer cells. Here, the jacalin was used to target the cell surface molecule Thomsen–Friedenreich carbohydrate antigen (T antigen), and the monoclonal antibody was specific to HER-2. Results from this study demonstrated that PDT toxicity *in vitro* was comparable between both conjugates in both cancer cell lines, illustrating that targeting carbohydrates with lectins and targeting transmembrane proteins with antibodies can elicit similar toxicities when combined with PDT. The authors suggest that this outcome may be explained by the different binding affinities and cell surface receptor densities. While the dissociation constant of jacalin binding to HT-29 cells is higher than that of anti-HER-2 antibodies binding to SK-BR-3 cells, the HT-29 expression of T-antigen is higher than the SK-BR-3 expression of HER-2 receptors. Thus, both binding affinity and target expression are critical parameters for the design of targeted nanotherapeutics.

Another common targeting moiety that is used to decorate the surface of nanoparticles is folate, which specifically targets folate receptors (332–334). The folate receptor is a GPI-anchored cell surface receptor that is overexpressed in many cancers, making it an attractive receptor for targeted therapies (342). The receptor is responsible for mediating the intracellular transport of folate via receptor-mediated endocytosis. In a study by Kato *et al.* (332), folate receptor-targeted porphyrins were synthesized and studied as a lung cancer treatment *in vitro* and *in vivo*. In an orthotopic lung tumor model, both the non-targeted porphyrins and folate-targeted porphyrins (FPs) had higher accumulation in the tumor compared to the healthy lung tissue, but FP administration led to higher intracellular PS accumulation. This study illustrates that nanoparticles with and without targeting moieties are able to accumulate preferentially at the tumor, but the targeting moieties are key for improving uptake by target cells.

Similarly, peptides can also be conjugated to the nanoparticle surface to achieve tumor-specific drug delivery. The use of peptides for targeting has several potential benefits over antibodies, including lower risk of immune reactivity, smaller size, high stability, and simple synthesis (343, 344). In a study by Sebak *et al.* (335), the peptide, cyclic (Arginine-Glycine-Aspartic acid-D-Tyrosine-Lysine) (cRGDyk), was conjugated to poly(lactic-co-glycolic acid) (PLGA) nanoparticles. The cRGDyk peptide served dual functions in this construct as both a targeting moiety and a quenching agent. Quenching agents like this are attractive features for PDT because they attenuate singlet oxygen generation outside of the tumor site, thereby improving specificity and limiting off-target cytotoxicity (345). In another study, Gao *et al.* (346) investigated the effects of  $\alpha\beta_6$  integrin-targeted PDT on host immune response. The authors demonstrated that integrin-targeted PDT using IRDye700-streptavidin-biotin-HK peptide (DSAB-HK) could both inhibit tumor growth and stimulate the host anti-tumor response by promoting dendrite maturation and T lymphocyte

recruitment. While PDT can be used to target integrins for cancer cell destruction, PDT has also been shown to modulate integrin expression in cancer cells. One study showed a significant reduction in the protein and mRNA expression of FAK and  $\beta 1$ -integrin 12 hours after PDT (347). Similarly, Runnels and colleagues have shown that BPD-mediated PDT at a mild dose ( $0.5 \text{ J cm}^{-2}$ ) induces the loss of  $\beta 1$  integrin-containing focal adhesion plaques (162). Because BPD was shown to localize in the mitochondria with no marked fluorescence at the membrane, and  $\beta 1$ -containing integrins seemed to be structurally intact after photosensitization, the authors suggest that the PDT-mediated loss of integrin function occurred through intracellular damage rather than direct photodamage.

While the vast majority of targeted PDT strategies have focused on targeting cancer cells, there are a variety of stromal cells that contribute to cancer progression, as discussed in a previous section. TAMs are known to promote cancer proliferation and metastasis through a variety of mechanisms including inflammatory cytokine release, increased angiogenesis, suppression of T cell response, and ECM remodeling (348, 349). Due to the role of TAMs in ECM remodeling, strategies that target these tumor-promoting immune cells could potentially impact the stiffness of the TME.

Previous work by Hayashi *et al.* (329) has demonstrated that PDT can be used to target TAMs. The authors synthesized mannose-conjugated chlorin (M-chlorin) to target the overexpressed mannose receptors on TAMs (Figure 8b). While these constructs were initially designed for specific toxicity in TAMs and not cancer cells, it was found that M-chlorin-PDT conferred cytotoxicity to tumor cells to a similar degree *in vitro* as the cancer cell-targeted control, glucose-conjugated chlorin (G-chlorin). This was attributed to the expression of mannose receptors on cancer cells. However, *in vivo* studies showed that M-chlorin PDT led to significantly more tumor suppression than G-chlorin PDT, which was attributed to the affinity of M-chlorin for CD-206 expressing TAMs in addition to cancer cells. These results suggest exciting opportunities for future studies that target multiple cell types with a single targeting moiety to achieve enhanced anti-tumor effect. Such constructs could potentially achieve synergistic cytotoxicity by killing cancer cells as well as the non-malignant cells that contribute to tumor maintenance, thereby providing an inherent mechanism for managing the cancer cells that survive initial treatment. Similarly, Wen and colleagues used a natural noninfectious nanocarrier, cowpea mosaic virus (CPMV), to target TAMs (74). CPMV has been shown to have a preference for M2-polarized macrophages. However, they also have affinity for vimentin, which is overexpressed in certain epithelial cancers, including prostate cancer, gastrointestinal tumors, breast cancer, and lung cancer (350). According to the results of the study, the PS-CPMV nanoconstruct (zinc ethynylphenyl porphyrin conjugated CPMV/ dendron hybrid) was effective on both cancer cells and macrophages but did not favor the M2 subpopulation, despite previous work suggesting that CPMV has a preference for M2-polarized macrophages (74).

## CONCLUSION

PDT- and PIT-based treatments and combination regimens provide several advantages over conventional agents. These advantages include distinct cytotoxic mechanisms and dual selectivity achieved via both spatiotemporal control of light and PS targeting. An area that

remains understudied in the design of targeted PDT and multi-agent combinations is the role of mechanical stress and the stromal components in the TME. This can include the destruction of cellular components such as CAFs and TAMs or degradation of noncellular components to modulate mechanical stresses and alter the TME. Controlled alterations in the tumor architecture and stiffness could enhance cytotoxic effects due to the role of the TME in promoting a variety of survival pathways in tumors (351).

Previous studies exploring targeting strategies against CAFs and TAMs, including by PDT, (321–324), (336), (348, 349) have demonstrated the potential to regulate the TME and modulate stiffness. These findings have implications for enhanced immune response (e.g. T cell infiltration) and drug delivery (321–324). Further work could explore how PDT-mediated targeting of CAFs and TAMs impacts metastatic potential and cell motility by remodeling the TME. This review also addresses the potential of PDT to modulate noncellular components of tumor ECM, such as collagen and hyaluronic acid. Although remodeling of the ECM seems promising for decreasing mechanical stress in the TME, the degradation of such components may also have unwanted outcomes, such as promoting invasiveness and migration of cancer cells, emphasizing the need for improved understanding and careful design of targeted regimens.

This review divides targeted PDT into three main categories: *targeting through by cellular and tissue modulation*, *functional targeting*, and *targeted delivery*. However, many of the categories can be combined to cooperatively achieve improved treatment outcomes depending on the target site. For example, many nanoparticle treatments that require intravenous injection will likely accumulate at the tumor site passively due to the EPR effect, underscoring the critical role that *functional targeting* can have for a variety of cancer treatments. Additionally, *cellular and tissue modulation* strategies can be utilized to prime a tumor for another targeted treatment strategy to enhance PDT efficacy. Therefore, the future of targeted PDT is not necessarily in one singular strategy, but rather the development of highly targeted treatment plans that leverage multiple routes to achieve specific and potent drug delivery.

Figure 9 summarizes the complexity of the relationships between mechanical stress and the TME components that have been reviewed here. Many components of the TME and mechanical stress are inter-related, highlighting the importance of rationally-designed targeted PDT combination that exploit and modulate the TME with the goal of enhanced tumor destruction and inhibition of metastases. Future work could also focus on using PDT to target other cell types that are present in the stroma and implicated in promoting tumor growth and metastasis. Strategies that simultaneously target cancer cells and the tumor-promoting noncancerous cells have been explored as well (329), and there is immense potential for further studies in this area.

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## ABBREVIATIONS

<b>5-ALA</b>	5-aminolevulinic acid
<b>AMD</b>	age-related macular degeneration
<b>BPD</b>	benzoporphyrin derivative
<b>CAF</b>	cancer-associated fibroblast
<b>CNV</b>	choroidal neovascularization
<b>CPMV</b>	cowpea mosaic virus
<b>CPO</b>	coproporphyrinogen
<b>DMA</b>	2,3-dimethylmaleic anhydride
<b>ECM</b>	extracellular matrix
<b>EGFR</b>	epidermal growth factor receptor
<b>EMT</b>	epithelia-mesenchymal transition
<b>EPR</b>	enhanced permeability and retention
<b>FAP</b>	fibroblast-activation protein
<b>FDA</b>	U. S. Food and Drug Administration
<b>FECH</b>	ferrochelatase
<b>HIF</b>	hypoxia-inducible factor
<b>IFP</b>	interstitial fluid pressure
<b>L-PDT</b>	low-dose photodynamic therapy
<b>MMP</b>	matrix metalloproteinase
<b>NLS</b>	nuclear localization sequence
<b>NMAPS</b>	nonmembrane anchoring photosensitizer
<b>OVV</b>	oncolytic vaccinia virus

<b>PDT</b>	photodynamic therapy
<b>pHLIP</b>	pH low insertion peptide
<b>pHMAPS</b>	pH-driven membrane anchoring photosensitizer
<b>PIC</b>	photoimmunoconjugate
<b>PIT</b>	photoimmunotherapy
<b>PpIX</b>	protoporphyrin IX
<b>PS</b>	photosensitizer
<b>PTT</b>	photothermal therapy
<b>TAM</b>	tumor-associated macrophage
<b>TGF-<math>\beta</math></b>	transforming growth factor- $\beta$
<b>TME</b>	tumor microenvironment
<b>TPZ</b>	tirapazamine
<b>VEGF</b>	vascular endothelial growth factor
<b>ZnPc</b>	Zinc phthalocyanine

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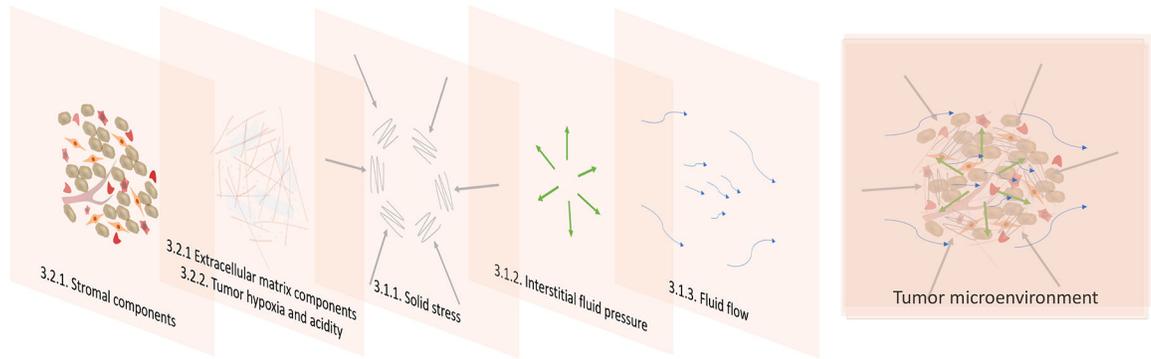
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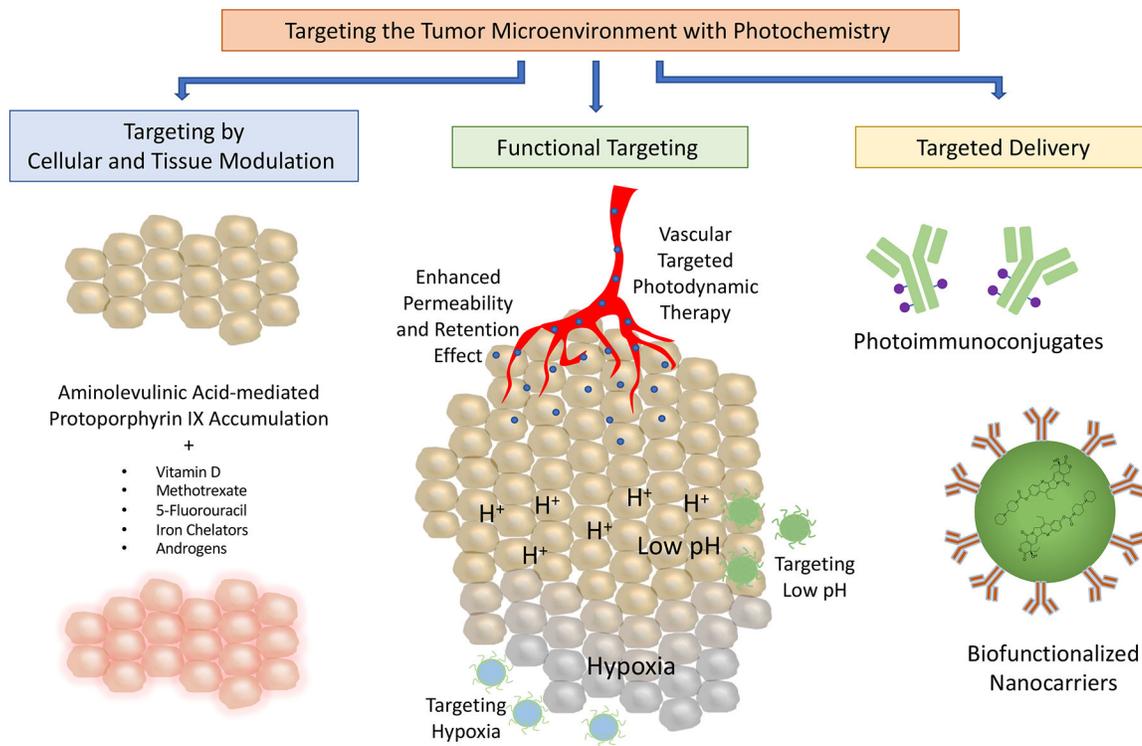
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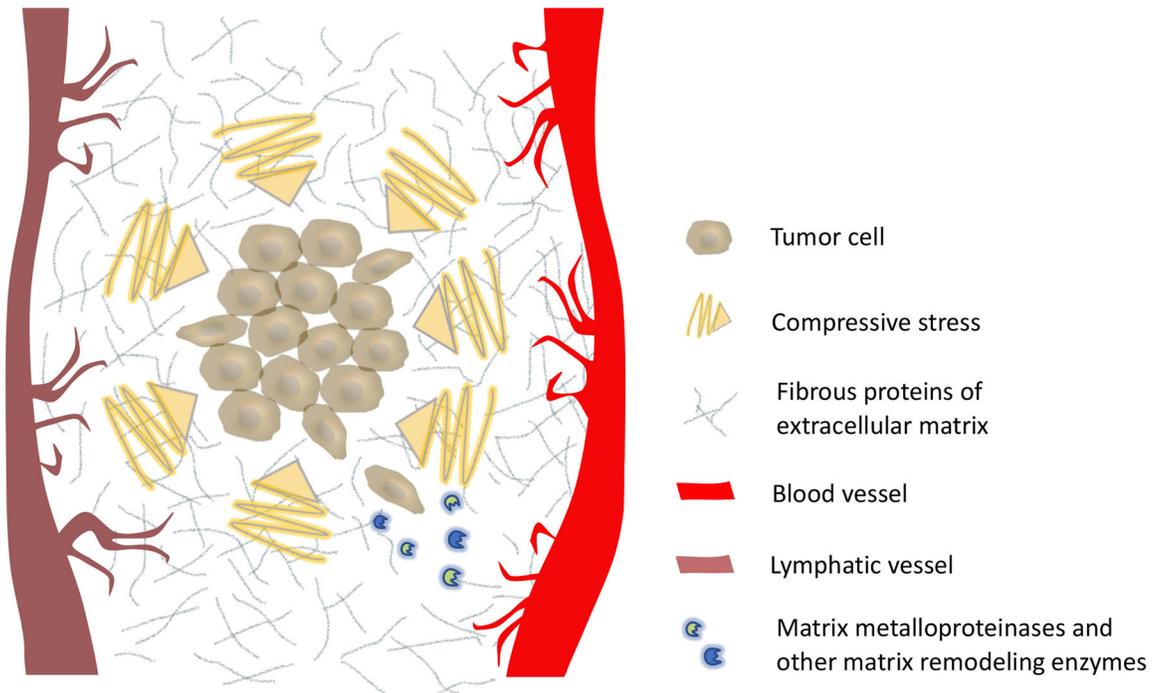


**Figure 1.**

The tumor microenvironment is composed of cellular and noncellular components, as well as various mechanical stresses, that can inhibit or promote tumor growth and survival. These stromal components and mechanical stresses are described in the section on the tumor microenvironment as a target for cancer treatment.

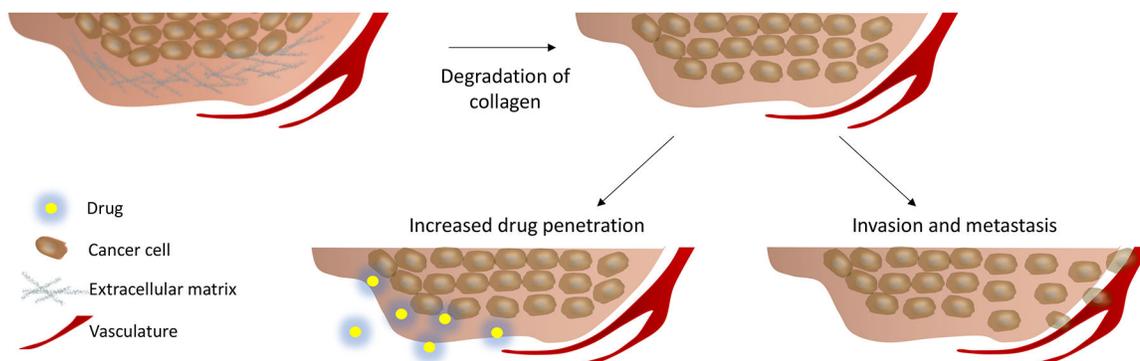


**Figure 2.** Categories of targeted photodynamic therapy that exploit cellular, molecular, and mechanical features of the tumor microenvironment: targeting by cellular and tissue modulation, functional targeting and targeted delivery.



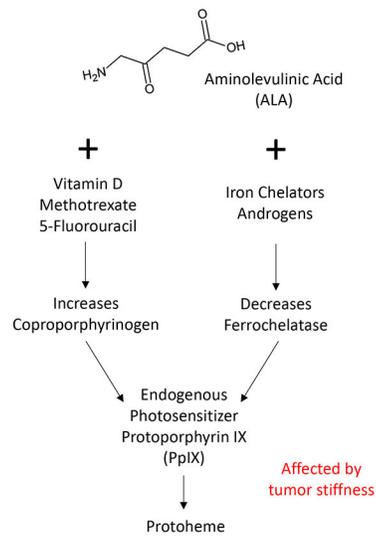
**Figure 3.**

The stiff extracellular matrix in the tumor microenvironment contributes to the generation of compressive stress on tumors. Compressive stress can inhibit tumor growth, while simultaneously activating invasive behavior. While dense, fibrous extracellular matrix impedes motility, invading cancer cells use matrix metalloproteinases, or other matrix remodeling enzymes, to create migratory paths (50).

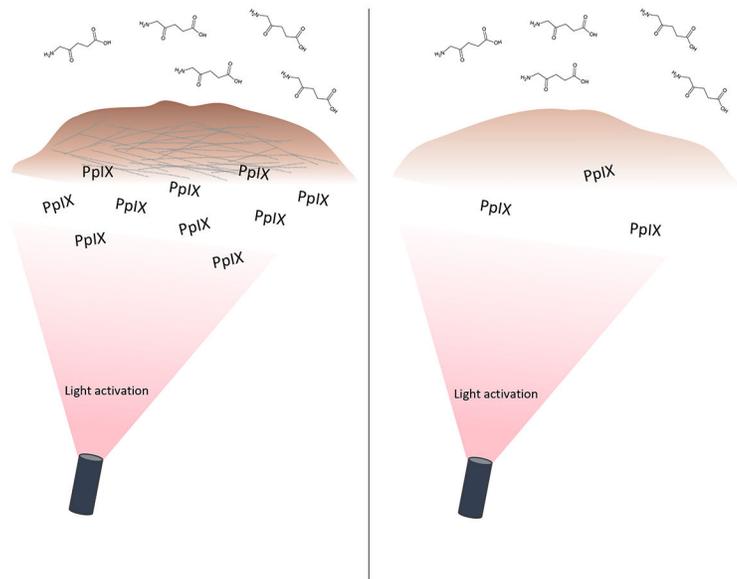


**Figure 4.** Extracellular matrix degradation can increase drug penetration into tumor tissues, though this degradation can also facilitate cancer cell invasion and metastasis.

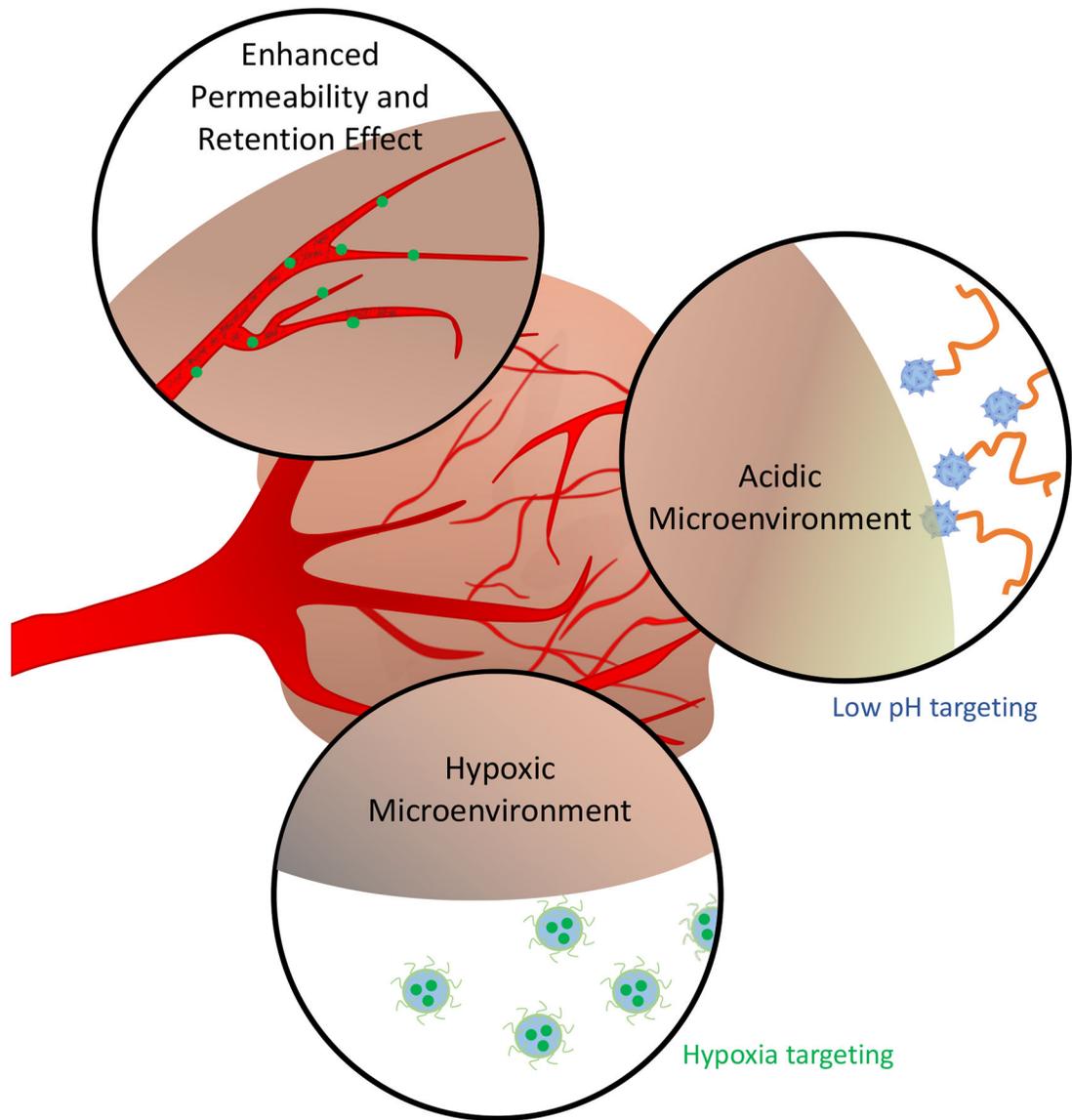
## a) Targeting by Cellular and Tissue Modulation



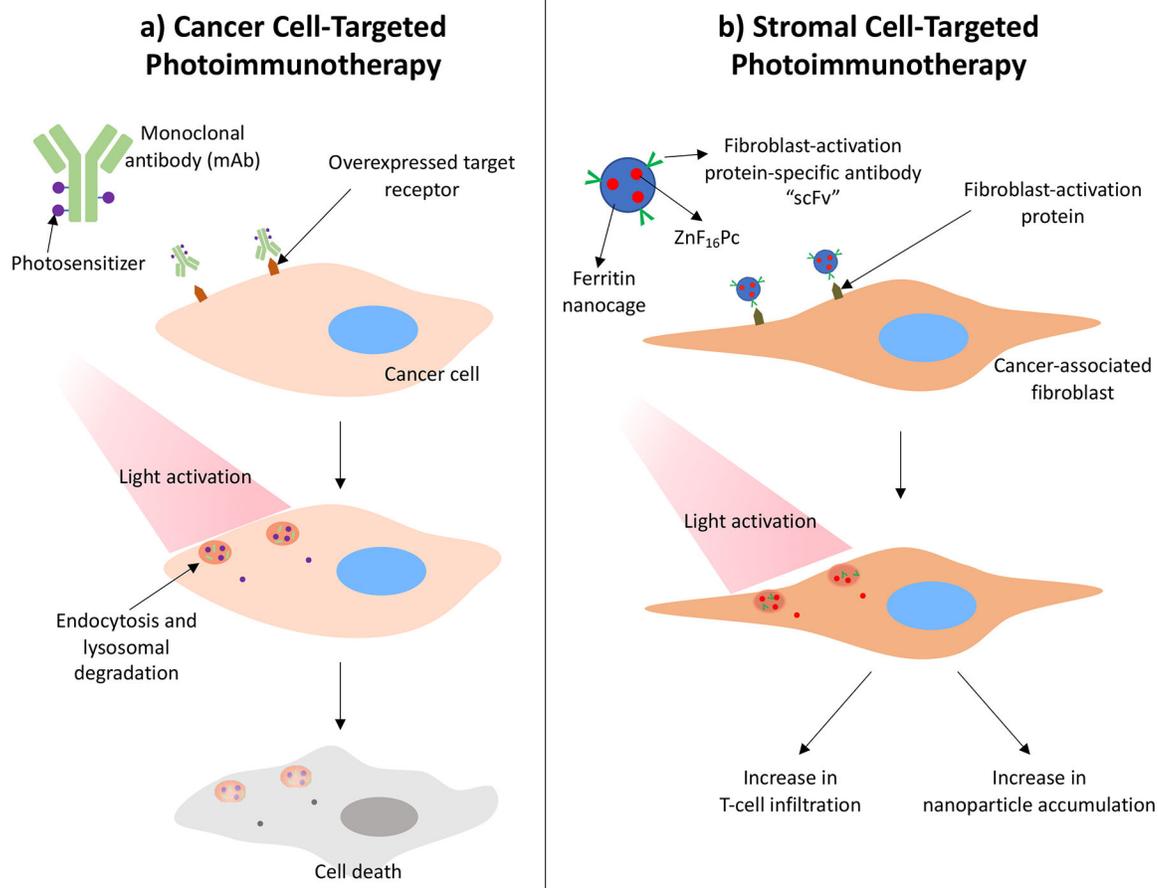
## b) Matrix Stiffness Affects Protoporphyrin IX (PpIX) Production

**Figure 5.**

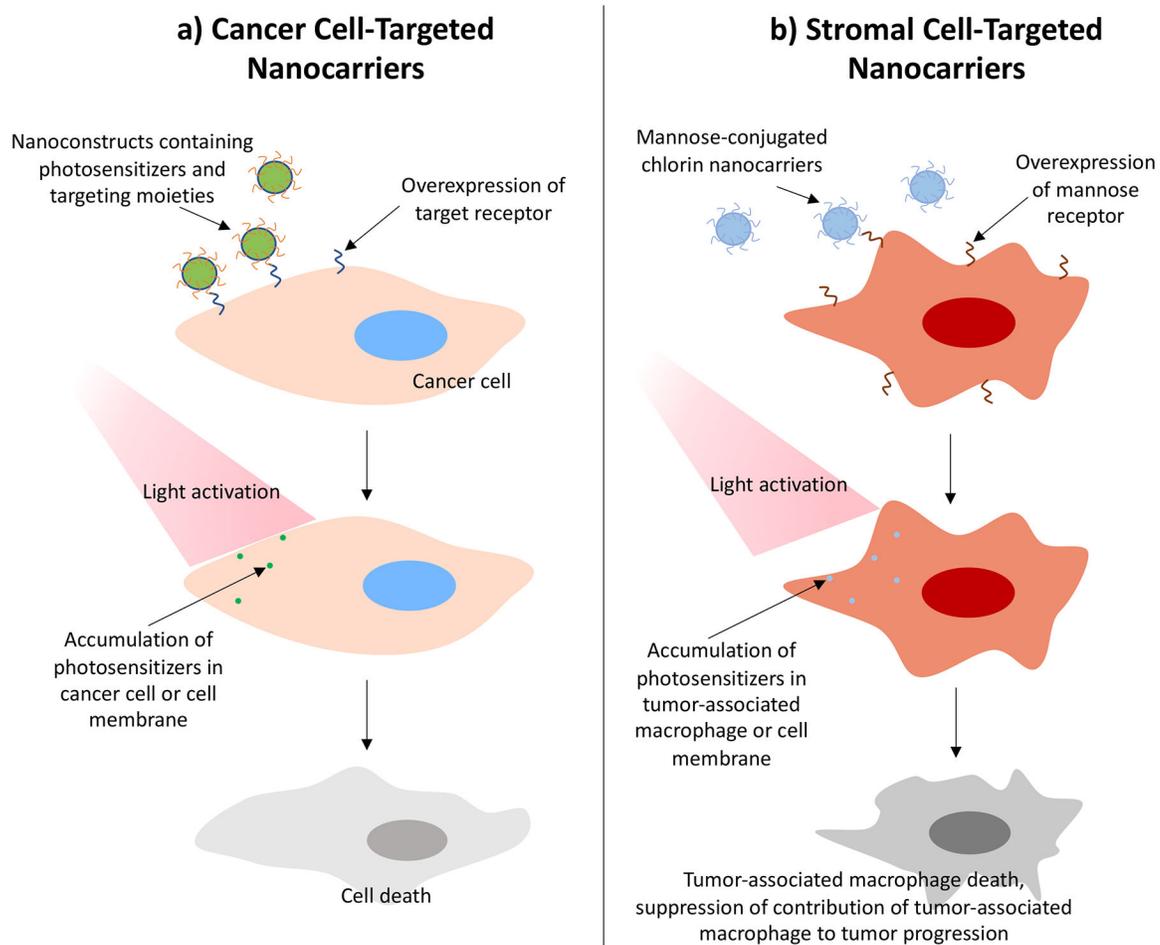
a) Summary of the pathways involved in targeting by cellular and tissue modulation, and b) increased PpIX accumulation with higher substrate stiffness in glioma cells (13).



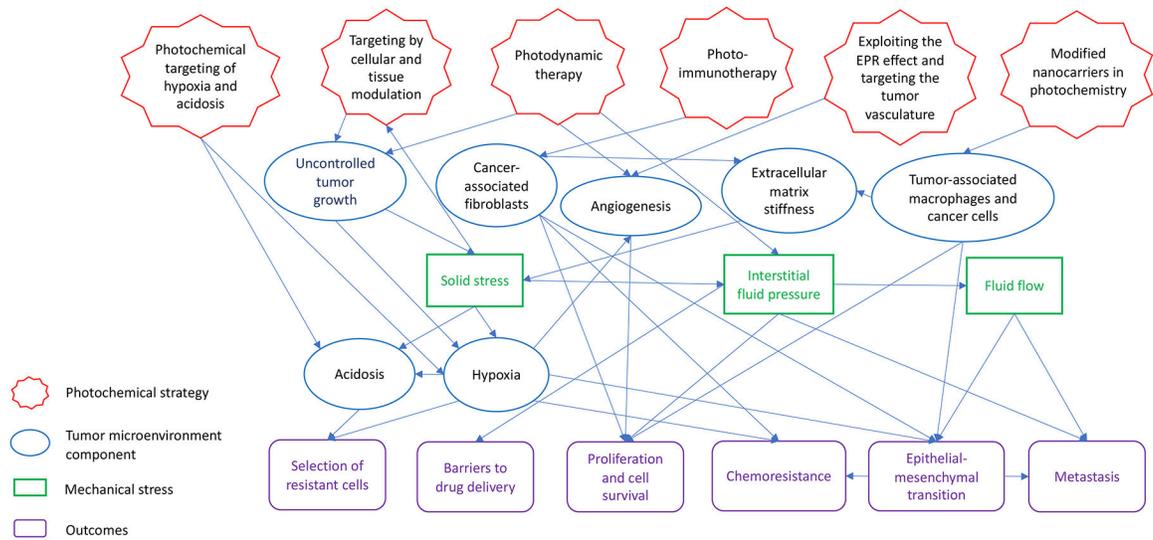
**Figure 6.** Characteristics of the tumor microenvironment that are exploited for functional targeting.

**Figure 7.**

a) Schematic of photoimmunoconjugate uptake by a cancer cell. The antibody binds to the target cell and is internalized by receptor-mediated endocytosis. At certain photosensitizer:antibody ratios, lysosomal degradation mechanisms can be leveraged to dequench the photosensitizer, allowing for light activation. Following light activation, reactive molecular species are generated to induce cytotoxicity in target cell. b) Cancer-associated fibroblast-targeted photoimmunotherapy and the associated effects on the tumor microenvironment (321, 324).



**Figure 8.** Depiction of the interactions between targeted photoactivatable nanocarriers and a) cancer cells or b) tumor-associated macrophages (329).



**Figure 9.**

The web of interactions between the components and mechanical stresses of the tumor microenvironment, and how they contribute to the development of treatment resistance and cancer progression. Photodynamic therapy can be used to alter these outcomes.

**Table 1.**

Photosensitizers that are commonly used in the clinic and in preclinical studies.

Photosensitizer	Family	Generation	Localization	References
HpD	Porphyrin	1 <sup>st</sup>	Multiple organelles and cell membrane	(160, 170)
BPD	Porphyrin	2 <sup>nd</sup>	Mitochondria and ER, or vasculature depending on the photosensitizer-light interval	(161, 162)
NPe6	Chlorin	2 <sup>nd</sup>	Lysosomes	(132, 168)
Tookad	Bacteriochlorin	2 <sup>nd</sup>	Tumor vasculature	(163)
Photofrin	Porphyrin	1 <sup>st</sup>	Plasma membrane and Golgi apparatus	(164)
Foscan	Chlorin	2 <sup>nd</sup>	ER and Golgi apparatus	(165, 166)
ZnPc	Phthalocyanine	2 <sup>nd</sup>	Mitochondria and lysosomes	(167)
ALA	Porphyrin	2 <sup>nd</sup>	PpIX produced in mitochondria, some relocalizes to the plasma membrane and lysosomes	(169)

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**Table 2.**

A summary of differentiating agents, along with the target diseases.

Differentiating Agent	PpIX Accumulation Mechanism	Diseases Treated	References
Vitamin D	Increases coproporphyrinogen	breast cancer, squamous cell carcinoma, melanoma, actinic keratosis, prostate cancer	(191, 192, 203, 211–213)
5-Fluorouracil	Increases coproporphyrinogen	actinic keratoses, melanoma, cervical cancer, mucosal lesions, intraepithelial squamous cell carcinoma	(193–197)
Iron Chelators	Decreases ferrochelatase	lung and normal fibroblasts, epidermal, pancreatic, bladder, colon, urothelial and skin carcinoma, adenocarcinoma carcinomas, basal cell carcinoma, solar keratoses, and lesions of Bowen's disease	(198–202, 204–210)
Androgens	Decreases ferrochelatase	prostate cancer, breast cancer	(211, 214)
Methotrexate	Increases coproporphyrinogen	prostate cancer, buccal precancers, cervical cancer, head and neck cancer	(215–217)

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**Table 3.**

A summary of various types of targeting moieties and details regarding their use.

Targeting moiety	Nanoparticle or molecule to which the moiety is conjugated	Target receptor	Target disease	References
Folic acid	Porphyrin-lipid nanoparticles (porphysomes), Hollow mesoporous silica nanoparticles, PLGA nanoparticles,	Folate receptor	Lung cancer, Skin cancer, Colorectal cancer	(332–334)
Peptides	Gold nanoparticles, PGLA nanoparticles	T antigen, $\alpha_v\beta_3$ , $\alpha_v\beta_6$ , EGFR	Colon cancer, Breast cancer, Melanoma, Glioma	(331, 335, 336)
Antibodies	Gold nanoparticles, Liposomes, PLGA nanoparticles	HER-2, EGFR	Colon cancer, Breast cancer, Epidermoid carcinoma, Ovarian cancer, Glioblastoma	(325, 331, 337, 338)
Carbohydrates	Chlorin, Nanomicelles, Gold nanoparticles, PLGA nanoparticles	Mannose receptor, Galectin-1, CD44	Gastric cancer, Colon cancer, Breast cancer, Oral squamous cell carcinoma	(327–330)