Review article

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Enhanced nanodrug delivery in tumors after near-infrared photoimmunotherapy

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Abstract: To date, the delivery of nanosized therapeutic agents to cancers largely relies on the enhanced permeability and retention (EPR) effects that are caused by the leaky nature of cancer vasculature. Whereas leaky vessels are often found in mouse xenografts, nanosized agents have demonstrated limited success in humans due to the relatively small magnitude of the EPR effect in naturally occurring cancers. To achieve the superior delivery of nanosized agents, alternate methods of increasing permeability and retention are needed. Near-infrared photoimmunotherapy (NIR-PIT) is a recently reported therapy that relies on an antibody-photon absorber conjugate that binds to tumors and then is activated by light. NIR-PIT causes an increase in nanodrug delivery by up to 24-fold compared to untreated tumors in which only the EPR effect is present. This effect, termed super-EPR (SUPR), can enhance the delivery of a wide variety of nanosized agents, including nanoparticles, antibodies, and protein-binding small-molecular-weight agents into tumors. Therefore, taking advantage of the SUPR effect after NIR-PIT may be a promising avenue to use a wide variety of nanodrugs in a highly effective manner.

Keywords: cancer; near-infrared photoimmunotherapy; EPR effects; super-enhanced permeability and retention (SUPR) effects; nanodrug delivery.

1 Introduction

Cancer is a leading cause of death in the world and the incidence is gradually increasing. An estimated 17.2 million cancer cases and 8.9 million cancer deaths worldwide occurred in 2016 [1]. The most common cancer treatments available today are surgery, radiation therapy, chemotherapy, and immunotherapy. Surgery and radiation therapy are useful for local and nonmetastatic carcinomas, but they are not effective for distantly spread tumors. In contrast, chemotherapy and immunotherapy are the only available treatment options for unresectable or metastatic tumors. Drug delivery is an important issue in treating cancers with systemic therapy.

Conventional low molecular anticancer drugs (molecular weight <1000) often lack tumor selectivity and distribute nonspecifically throughout the body, leading to toxic side effects in normal tissues. Unbound drug is rapidly cleared from the circulation, so the patient must be given relatively large doses to achieve the required therapeutic concentration in the cancer. This exacerbates off-target effects. Larger molecules often fail to reach significant concentrations within the tumor due to the modest enhanced permeability and retention (EPR) effect and the longer circulation times contribute to off-target effects.

Compared to the conventional small or large single-molecule anticancer drugs, the development of a drug delivery system that enables tumor-targeted drug delivery could overcome some of these limitations. Compared to conventional anticancer agents, nanosized drugs have a number of advantages, including a large loading capacity, the ability to protect the payload from degradation, specific targeting, and controlled or sustained release. These features can be modified by changing the characteristics such as the size, shape, payload, and surface features. Thus, nanomedicine-based drug delivery systems have been actively investigated as a means of improving drug effectiveness. Overcoming the delivery barrier, however, remains a challenge for the field of nanomedicine. Near-infrared photoimmunotherapy (NIR-PIT) represents a potential solution to some of these challenges, as

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it induces a tumor-specific super-EPR (SUPR) effect that preferentially allows nanosized drugs into the tumor.

In this review, we first overview the challenges of delivering nanosized drugs into cancer tissue, including the limitations of passive targeting, active targeting, and triggered drug release. Then, we discuss how the EPR effect can be improved and end with a discussion of the SUPR effect induced by NIR-PIT.

2 Challenges of delivering nanodrugs into tumors

There are three main mechanisms by which nanodrugs can enter a tumor bed: passive targeting, active targeting, and triggered drug release (Figure 1A). Passive targeting depends on the leakage of the nanodrug due to increased vascular permeability and the retention due to poor venous and lymphatic drainage. Active targeting relies on functionalizing the nanodrug surface, thereby causing increased binding of the nanodrug to tumor cells versus normal cells. Triggered drug release occurs when there is activation and release of the drug from nanoparticles that have reached target sites in response to intrinsic or extrinsic stimuli.

2.1 Passive targeting

An advantage of nanodrugs over small-molecular-weight drugs is that compared to normal tissue they preferentially leak and are retained in tumors. This EPR effect, first described by Matsumura and Maeda in 1986, is the basis for passive targeting [3, 4].

Solid tumors often possess a leaky vasculature compared to normal tissues. The endothelial surface is fenestrated with gaps between endothelial cells and is surrounded by abnormal basement membranes and fewer or poorly adherent pericytes. As a result, nanodrugs can more easily extravasate and accumulate within the interstitial space of tumors. In addition, solid tumors often lack of functional lymphatics, leading to the impaired clearance of leaked drugs. Consequently, nanodrugs tend to be retained in tumors, whereas small molecules more easily diffuse in and out of tumors and normal tissues with equal ease. The magnitude of the EPR effect varies between spontaneously formed and implanted tumors, typically found in xenograft mouse models. The latter tend to be highly leaky and exaggerate the preferential leakage on nanodrugs in animal models. The vasculature of naturally

occurring human tumors, although abnormal, is not as leaky.

Nevertheless, the EPR effect can be amplified by the appropriate choice of particle size, particle charge, and hydrophobicity, which can prolong circulation kinetics, thereby increasing the likelihood of intratumor uptake [5, 6]. Hydrophobic nanoparticles are frequently associated with serum proteins and metabolized in the liver. Positively charged nanoparticles generally are more prone to recognition by the reticuloendothelial system and therefore are unavailable for tumor uptake [7, 8]. As the glomerular basement membrane in the kidney is negatively charged, the net charge of nanoparticles highly influences renal excretion [9–11]. Nanodrugs <5 nm in diameter are quickly eliminated via renal filtration, whereas those with a size of >200 nm tend to accumulate in the liver, spleen and lungs. Thus, practical nanoparticles are limited in size (probably to <30 nm in diameter) and are often coated with neutral hydrophilic polymers, such as polyethylene glycol (PEG) and polysaccharides [12, 13]. To improve the therapeutic effects of nanodrugs, these physiological parameters have to be optimized. The tumor-related barriers to passive targeting are discussed in Section 3.1.

2.2 Active targeting

Passive targeting by the EPR effect only facilitates the localization of nanodrugs into the extracellular space within tumors and not within target cells. To increase the uptake of nanodrugs in cancer cells, active targeting is required. Active targeting depends on the specific recognition and binding of the nanodrug to a target site on the cancer cell surface. Frequently used targeting ligands are monoclonal antibodies and their fragments, lectins, aptamers, peptides, and other small molecules [14–17]. As antibodies have high selectivity and binding affinity for their corresponding antigens, they are commonly employed as antibody-drug conjugates or antibody-isotope conjugates, such as Ontak®, Zevalin®, and Bexxar®, which have been approved for clinical use [18, 19].

The binding of ligands to their cognate receptor leads to receptor-mediated internalization, which may help to overcome drug resistance by internalizing the drug. Cancer drug resistance is often due to the overexpression of multidrug-resistant transporters that expel drugs from cancer cells. Goren et al. reported that folate receptor-mediated cellular uptake of liposomal doxorubicin was not affected by P-glycoprotein-mediated drug efflux [20]. This feature is also useful for transporting reagents that

Figure 1: How to improve nano-delivery into tumor beds beyond intrinsic EPR effects.

(A) Schematic view of nanodrug delivery. Passive targeting is achieved by the leakage of nanodrugs due to increased vascular permeability and retention due to poor venous and lymphatic drainage (EPR effect). Active targeting (inset) is accomplished by functionalizing the nanodrug surface, leading to increased binding of the nanodrug to target cells. Reprinted by permission from Springer Nature: Peer D et al. "Nanocarriers as an emerging platform for cancer therapy" Nature Nanotechnology. Copyright 2007. (B) Strategies for improving the EPR effects by modifying intrinsic physiological conditions: (1) modulation of tumor blood flow using vasoconstrictor or vasodilator, (2) modulation of tumor vasculature and stroma, and (3) elimination of tumor cells. Adapted from Ref. [2].

would otherwise be unable to enter cancer cells by themselves, such as nucleic acids and proteins [21, 22].

Although the higher binding affinity of antibodies is generally considered a favorable feature and usually increases efficacy, excessive affinity can result in a phenomenon known as the binding-site barrier. In this situation, high-affinity nanoparticles cannot penetrate deeply into tumors because they are easily trapped at their first encounter with the receptor and saturating doses are not usually given [23].

Although active targeting has obvious appeal as a delivery strategy, the targeted agent must first enter the tumor interstitium to be effective. Accordingly, adequate passive targeting, subject to all the limitations discussed above, needs to be achieved before active targeting can be effective.

2.3 Triggered drug release

Triggered drug release has the obvious appeal that the therapy is only activated when it has reached the tumor and will not be active outside the tumor unless activated. Nanoparticles that can be triggered to release their payload in response to intrinsic or extrinsic stimuli are thus considered “activatable” nanodrugs [24].

Several triggers can be used to release drugs from their nanocarrier. For instance, the lower extracellular pH found in the tumor microenvironment can be an excellent trigger. Tumor cells often employ aerobic glycolysis, often referred to as Warburg physiology, to metabolize glucose, leading to the accumulation of lactic acid, a by-product of glycolysis. Furthermore, intracellular organelles such as endosomes and lysosomes exhibit low pH; thus, endocytosed nanodrugs are subject to progressively lower pH conditions. pH-responsive nanoparticles rely on pH-sensitive linkage or pH-dependent conformational changes [25–27]. Redox-sensitive polymers can also be used to release drugs. Glutathione is found in relatively high concentrations in the tumor intracellular environment compared to the extracellular environment. Using glutathione-mediated disulfide bond cleavage, redox-sensitive nanodrugs can be assembled [28, 29].

Another potential trigger for drug release is temperature. For instance, temperature-sensitive liposomes (TSLs) have been developed that release encapsulated drugs upon heating. Details are provided in Section 4.2. Another potential trigger for drug release is light which photochemically alter the nanocarrier to release drugs upon irradiation of light of a specific wavelength. Earlier studies have reported ultraviolet or visible light-responsive

polymers [30, 31]. A disadvantage of light activation is that light penetration is limited in tissue to a few millimeters unless infrared light or NIR light is used which has better tissue penetration due to lower absorption [32, 33].

3 EPR effects: limitations

3.1 Barriers for nanoparticle delivery

The main barriers to nanodrug delivery to the tumor micro-environment are the degree of leakiness of the tumor vasculature, high interstitial fluid pressure (IFP) within the tumor and solid stress. All of these factors impede delivery of a nanodrug to the extracellular tumor space. Abnormal tumor vasculature is present in most tumors and contributes to the EPR effect. Tumor vasculature lacks the conventional hierarchy of blood vessels; arterioles, capillaries, venules are not recognizable as such, and rather, vessels are enlarged and often interconnected with bidirectional shunts [34]. Such conditions lead to heterogeneity of tumor blood flow, which inhibits the homogenous distribution of nanodrugs.

Blood vessel leakiness allows extravasation of excessive fluid and plasma macromolecule into the tumor interstitial tissue. Furthermore, poor lymphatic drainage in tumors elevates IFP. In normal tissues, IFP is in the range of 0–3 mm Hg; whereas in murine and human tumors, IFP reaches from 10 to 40 mm Hg [35]. High IFP impairs blood flow by mechanical compression of tumor vessels, reducing the vascular transport of nanoparticles. Moreover, high IFP inhibits drug delivery by convection, while paradoxically promoting passive diffusion out of tumors [36].

The dense composition of extracellular matrix (ECM) and proliferating cancer cells creates another form of barrier known as solid stress. Solid stress hinders nanodrug delivery by compressing tumor vessels. In addition, the dense and highly cross-linked ECM network directly limits the diffusion of nanodrugs. The interstitial diffusion of nanoparticles larger than the mesh size of the stroma is reduced [37]. ECM is much more abundant in human tumors compared to experimental tumors, further reducing the EPR effect.

As mentioned previously, as nanodrugs are designed to be larger, they are more apt to be recognized and taken up by macrophages after crossing tumor blood vessels. In theory, macrophages having taken up a nanodrug could migrate and deliver drugs into a tumor; in reality, this is not sufficiently reliable to be effective.

3.2 Disappointing clinical outcomes with EPR-based drug delivery

There are often some discrepancies between animal experiments and human clinical treatments. This is especially true for the EPR effect where hopeful effects seen in mouse xenografts lead to disappointing results in humans [38].

To be clear, there have been some nanodrugs that have successfully used EPR. Doxil[®], a pegylated liposomal doxorubicin, was the first U.S. Food and Drug Administration (FDA)-approved nanosized drug carrier. In preclinical trials, pegylated liposomal doxorubicin showed reduced toxicity and superior efficacy over free doxorubicin. In phase 3 trials for AIDS-related Kaposi's sarcoma and ovarian cancer, Doxil[®] showed superior therapeutic efficacy compared to standard therapy [39, 40]. In contrast, Doxil[®] did not improve overall survival over conventional doxorubicin in metastatic breast cancer. Moreover, although Doxil[®] reduced cardiotoxicity, it increased the incidence of hand and foot syndrome due to the longer circulation time [41].

Nab-paclitaxel (Abraxane[®]) is another nanoformulation composed of albumin-bound paclitaxel [42, 43]. Nab-paclitaxel showed significantly improved clinical outcomes compared to paclitaxel. As paclitaxel is very hydrophobic, it must be solubilized in polyoxyethylated castor oil (Cremophor EL[®]). Much of the toxicity of Taxol[®] owes to Cremophor EL[®]. This excipient is associated with side effects such as bronchospasms, hypotension, and hypersensitivity reactions. As Abraxane[®] increases hydrophilicity by binding paclitaxel to albumin, it is not necessary to use Cremophor EL[®]. Therefore, the maximum tolerated dose of Abraxane[®] is 50% higher than Taxol[®]. There is a possibility that the higher therapeutic effect of Abraxane[®] may be associated with this ability to increase the dose rather than the EPR effect [44].

4 Strategies for improving the EPR effect

The efficiency of the EPR effect depends on several factors, including the presence of abnormal tumor vasculature, level of IFP, and degree of solid stress. By modifying any of these features, the EPR effect can be enhanced and more efficient nanodrug delivery can be achieved. There are three main methods of modulating EPR (Figure 1B) [2, 45]: (1) modulation of tumor blood flow using vasoconstrictor or vasodilator, (2) modulation of tumor vasculature and

stroma, and (3) elimination of tumor cells. As mentioned in the previous section, clinical outcomes of nanosized drug delivery systems are not as beneficial for patients as theoretically expected. Thus, novel methods for enhancing the EPR effect are potentially very important.

4.1 Modulation of tumor blood flow

4.1.1 Vasoconstriction

Tumor vasculature lacks a functional smooth muscle layer and has a reduced density of angiotensin-II (AT-II) receptors found normally in vessels [46]. Therefore, systemic hypertension induced by the intravenous administration of AT-II causes increased blood flow within tumors, whereas blood flow is constant in normal tissues [47]. Taking advantage of this feature, Li et al. have reported that AT-II-induced hypertension enhanced the selective delivery of styrene-maleic acid polymer conjugated to neocarzinostatin (SMANCS) in Walker 256 rat tumors while decreasing the distribution of SMANCS to normal tissues [48]. Even in patients with poorly vascularized solid tumors such as cholangiocarcinoma and liver metastasis in pancreatic cancer, AT-II-induced hypertension (110→150 mm Hg) enabled enhanced SMANCS/lipiodol delivery and resulted in improved therapeutic response, less toxicity, and a shorter time to achieve tumor regression [49].

4.1.2 Vasodilation

Another approach to improve the EPR effect is to use vasodilators. Nitric oxide (NO), produced from L-arginine and oxygen by NO synthase (NOS), causes the dilation of vessels and enhanced permeability. Whereas NO scavenger and NOS inhibitor inhibited the EPR effect, nitroglycerin ointment-derived NO enhanced macromolecular drug delivery in a dose-dependent manner in murine tumor models [50, 51]. In a phase II randomized control trial, the use of nitroglycerin combined with conventional low-molecular-weight anticancer drugs significantly improved the overall response and survival in patients with stage IIIB/IV non-small-cell lung cancer [52].

Bradykinin is a major inflammatory mediator produced by the kallikrein-kinin system in cancer tissue. Bradykinin is involved in cell proliferation, migration, angiogenesis, and increased vascular permeability [53]. Bradykinin also activates endothelial cell-derived NOS, leading to an increase in NO. Therefore, bradykinin affects vascular permeability directly and indirectly. The inhibition of

kinin-degrading enzymes (kinase I and kinase II) increased permeability up to 1.5-fold [54]. Conversely, the bradykinin B2 receptor antagonist, HOE 140, significantly suppressed the EPR effect in murine S-180 tumor [55].

Prostaglandins are physiologically active lipid compounds called eicosanoids that are derived enzymatically from arachidonic acid. Among various prostaglandins, PGI₂ inhibits platelet aggregation and is an effective vasodilator. The administration of PGI₂ analogue, beraprost sodium, increased the extravasation of Evans blue-albumin complex by twofold to threefold [56].

Carbon monoxide is generated from heme oxygenase-1 (HO-1) in the course of heme metabolism and exhibits various functions: vascular dilation, facilitation of blood flow, antioxidative effects, and antiapoptotic effects. Fang et al. reported that the HO-1 inducer pegylated hemin enhanced Evans blue-albumin complex accumulation within tumors [57].

4.2 Modulation of tumor vasculature and stroma

4.2.1 Normalization of tumor vessels

The balance of proangiogenic and antiangiogenic factors affects the formation of the tumor vascular architecture. Whereas the balance of these factors is maintained in normal tissues, proangiogenic factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are usually overexpressed in cancerous tissues, leading to the chaotic blood vessel formation [58]. Therefore, proper dosage of antiangiogenic drugs causes the normalization of tumor vasculature. Blockade of VEGF by a single infusion of bevacizumab (Avastin®) decreased microvascular density and IFP in colorectal patients [59]. The reduction of IFP allows a deeper penetration of anticancer drugs into tumors. However, it is notable that vascular normalization also means that there is an undesired decrease of vessel permeability. Moreover, the period of normalization can be short and unpredictable. Therefore, only smaller nanosized drugs profit from vascular normalization. Chauhan et al. reported that the anti-VEGF receptor 2 antibody DC101 led to threefold delivery enhancement for 12 nm particles with no improvement for the larger particles (60 and 125 nm) in murine 4T1 and E0771 mammary tumors [60]. Caution should be exercised when extending this preclinical experience to human patients. Jiang et al. showed that intermediate-sized nanoparticles (20–40 nm) also derived a benefit from DC101-mediated tumor vasculature remodeling [61]. Despite these

basic research findings, the combination of liposomal doxorubicin (100 nm) and bevacizumab for platinum-resistant ovarian cancer presented very positive outcomes [62].

4.2.2 Normalization of tumor matrix

Abnormal ECM composition and structure are major obstacles for nanodrug delivery in solid tumors. Hence, the modification of the ECM has been explored as a method of improving the distribution of nanodrug delivery. To date, two approaches to ECM modification have been attempted: ECM degradation and reduction of ECM synthesis by the inhibition of cancer-associated fibroblasts (CAFs).

Several studies have shown that ECM-degrading enzymes such as collagenase and hyaluronidase can improve the distribution of nanoparticles in a tumor. Collagenase-coated nanoparticles increased tumor penetration up to fourfold compared to albumin-coated control particles [63]. Intratumoral injection of collagenase was shown to induce a 10-fold increase in the diffusion of larger macromolecule, although such an approach is clearly unrealistic [64]. Another study showed that hyaluronidase increased the distribution of liposomal doxorubicin up to fourfold in human osteosarcoma xenografts [65]. Relaxin is a nontoxic hormone secreted during pregnancy that induces the up-regulation of various matrix metalloproteinases. Brown et al. have visualized the relaxin-mediated degradation of collagen fiber using second harmonic generation imaging [66]. Although relaxin was not as efficient as direct enzymatic treatment, it also enhanced diffusive transport. A phase 3 clinical trial using the combination of pegylated recombinant human hyaluronidase (PEGPH20) and chemotherapy is ongoing in metastatic pancreatic ductal carcinoma patients (<https://clinicaltrials.gov/ct2/show/NCT02715804>) [67].

CAFs play a major role in ECM production. Resting fibroblasts are transformed into CAFs by cancer-associated growth factors such as transforming growth factor- β (TGF- β), stromal-derived factor-1, PDGF, and basic fibroblast growth factor (bFGF) [68]. Therefore, ECM degradation can be achieved by blocking these factors. The inhibition of collagen synthesis by TGF- β blocking antibody normalized tumor interstitial matrix and improved the intratumoral delivery of both low-molecular-weight chemotherapeutic drugs and nanoparticles [69]. Cyclopamine, an inhibitor of the Hedgehog signaling pathway, disrupted tumor extracellular fibronectin and enhanced the accumulation and penetration of nanoparticles up to 2.6-fold [70].

Another more controversial approach has been the targeted depletion of CAF. Loeffler et al. reported that the use of an oral DNA vaccine targeting fibroblast activation protein suppressed primary tumor growth and metastasis in murine colon and breast cancer models via CD8⁺ T-cell-mediated killing of CAFs. Moreover, tumor tissue of vaccinated mice revealed decreased collagen I deposition and enhanced uptake of chemotherapeutic agents [71]. In contrast, myofibroblast depletion in murine pancreatic cancer model while decreasing type I collagen deposition also promoted epithelial-to-mesenchymal transition and led to poor survival [72].

4.2.3 Sonoporation: drug delivery using ultrasound and microbubbles

While microbubbles are generally only used as contrast agents for contrast-enhanced ultrasound imaging, ultrasound and microbubble-mediated drug delivery, which is called sonoporation, has attracted attention in recent years. Ultrasound irradiation of previously injected microbubbles triggers stable and inertial cavitation effects such as microstreaming and shockwaves, leading to generate transient pores in the blood vessel walls, even the blood-brain barriers [73, 74].

Lin et al. reported that sonoporation increased the delivery of pegylated liposomal doxorubicin up to 1.5-fold in the CT-26 tumors [75]. Theek et al. showed that sonoporation doubled the accumulation of fluorophore-labeled liposome in two tumor models (A431 and BxPC-3), which are known to have a low baseline EPR effect [76].

Recently, Kotopulis et al. have performed the first clinical trial of sonoporation-mediated drug delivery. The safety and efficacy of gemcitabine with concurrent exposure to SonoVue[®] microbubbles and ultrasound were indicated in patients with inoperable pancreatic ductal adenocarcinoma [77, 78]. Median survival was found to improve from 8.9 to 17.6 months in combination with sonoporation. Sonoporation has been also studied in the field of the lysis of blood clots. A phase 3 randomized controlled trial of sonothrombolysis for acute ischemic stroke (CLOTBUST-ER) was performed, but this trial was stopped early because no clinical benefit was seen [79].

4.2.4 Hyperthermia

Mild hyperthermia (39–42°C) improves drug delivery via enhanced blood perfusion. Increased blood flow causes increased intravascular pressures and enlarged pore sizes between endothelial cell junctions, leading to drug

extravasation. Hyperthermia also increases cell membrane permeability and thereby increases cellular uptake [80, 81]. Kong et al. reported that hyperthermia (42°C) significantly increased the extravasation of 100 nm liposome in the SKOV3 tumor but not in normal vasculature. These results potentially enable tumor-specific drug delivery [82]. They also indicated that temperature dependency of hyperthermia induced nanoparticle extravasation from 40°C to 42°C [83]. Several phase 1/2 clinical trials, using the combination of hyperthermia and non-TSLs, for breast and ovarian cancers have been performed [84–86]. Although hyperthermia-based combination therapy seemed effective for breast cancer patients, it was not beneficial for ovarian cancer patients.

Hyperthermia-mediated drug therapy is still more effective in combination with TSL, as first proposed by Yatvin et al. [87–89]. TSL is triggered to release its contents upon exposure to hyperthermia. When TSLs pass through the zone of elevated temperature (cancer), the contents of TSL are quickly released and high local concentrations of payloads within tumor vasculature are achieved, leading to significant anticancer effects. ThermoDox[®] (temperature-sensitive pegylated liposomes containing doxorubicin) have been well studied and are commercially available. Hyperthermia is delivered by a combination of focused microwave and other heat sources. A phase 1/2 clinical trial in patients with locoregional recurrent breast cancer on the chest wall suggested positive efficacy [90].

4.3 Eliminating tumor cells

4.3.1 Radiation therapy

Radiation therapy has been reported to increase vascular leakiness via the up-regulation of VEGF and bFGF [91, 92]. Furthermore, cellular density within tumors decreases due to cytotoxicity of radiation, leading to reduced IFP [93]. These features should allow better accumulation of nanodrugs in cancer tissues. Nanosized molecules can enter irradiated tumors at a rate 2.2-fold higher than non-irradiated tissue [94]. However, such increases can be heterogeneous as tissue reacts to radiation in different ways. Additionally, radiation can damage the tumor vasculature shutting down blood flow, thus negatively affecting the delivery of nanodrugs [45].

4.3.2 Photodynamic therapy (PDT)

Tumor-targeted PDT is a clinically approved cancer treatment based on porphyrin-derived photosensitizers.

These compounds generate reactive oxygen species in the presence of oxygen and light [95]. The tumor vascular response can vary from transiently enhanced vascular permeability to vasoconstriction and total vascular occlusion [96]. Snyder et al. demonstrated that low fluence rate PDT enhanced vascular permeability without producing vascular collapse. Low fluence rate PDT increased the accumulation of Doxil in Colo 26 tumors up to 2.5-fold compared to control tumors [97]. Vascular-targeted PDT also enhances vascular permeability. Because of the microtubule depolymerization and the actin stress fiber formation of endothelial cells, intercellular space is extended and vascular leakiness is increased [98].

5 SUPR effects induced by NIR-PIT

5.1 What is NIR-PIT?

NIR-PIT is a newly developed cancer treatment that uses an antibody labeled with NIR photon-absorbing silicon phthalocyanine dye, IRDye700DX (IR700). An antibody-photon absorber conjugate (APC) specifically binds to antigen-expressing cells, and subsequent exposure to NIR light (~690 nm) selectively kills targeted cells [99–103]. The light can be delivered virtually anywhere in the body by employing fibro-optical diffusers that can be used through 20-gauge injection needles or catheters or endoscopy systems [104, 105]. The results of a phase 1/2 clinical trial in patients with inoperable head-and-neck cancer are promising (<https://clinicaltrials.gov/ct2/show/NCT02422979>) and the FDA “fast tracked” a global phase 3 clinical trial that is currently ongoing (<https://clinicaltrials.gov/ct2/show/NCT03769506>).

Due to its water solubility, the dye IR700 in itself does not have photosensitizing effects such as porphyrin- or phthalocyanine-derivatives that are used for PDT. However, when IR700 is conjugated to an antibody, it behaves like a photosensitizer. Recently, Sato et al. revealed the mechanism of cytotoxicity of NIR-PIT [106]. When the antibody-dye conjugate is exposed to NIR light, axial ligands are dissociated from the IR700 molecule causing it to become dramatically more hydrophobic. This leads to conformational changes to mAb-IR700-cell membrane complexes resulting in the formation of dimers or oligomers. Single-molecule atomic force microscopy showed that the exposure of NIR light causes the loss of the normal Y-shape of the antibody, which also increases in size (Figure 2A and B). The aggregation of

antibody-IR700 conjugate induces physical stress in the cell membrane, resulting in weakening of the membrane, bleb formation followed by cell bursting, and necrotic cell death. In this mechanism of cell killing, NIR-PIT can be clearly distinguished from conventional PDT, which uses cytotoxic singlet oxygen. Several reports have detailed the spatiotemporal morphological effects of PIT on the cellular membrane using three-dimensional low-coherent quantitative phase microscopy (3D LC-QPM; Figure 2C) [107–109].

5.2 Mechanism of the SUPR effect

As mentioned in the previous section, NIR-PIT only kills targeted cells without damaging adjacent normal cells, such as vascular endothelial cells. As APCs are delivered to the tumor through the bloodstream, tumor cells adjacent to the tumor vessels are exposed to the highest concentrations of APCs. After NIR irradiation, most of the initial cell killing occurs in the perivascular layer of tumor cells. Therefore, a potential space develops between the tumor vasculature and the remaining tumor cells resulting in increased vascular permeability. This allows nanodrugs to enter the treated tumor beds in dramatically increased concentrations. The dramatic increase in permeability and retention in NIR-PIT-treated tumors has been termed the SUPR effect [110, 111]. In addition, when the targets of the APC and the targets of the nanodrugs are the same, active targeting-type nanodrugs can penetrate deeper in tumors because the binding-site barrier has been eliminated by NIR-PIT. In other words, the SUPR effect can overcome the challenge of “the binding-site barrier” and enhance even actively targeted nanodrugs. When NIR-PIT effectively kills target cancer cells, SUPR effects always operate regardless of cancer cell types or nanodrugs because SUPR effects are based on this physical mechanism in the tumor microstructure.

The SUPR effect has been reported to increase in nanodrug delivery up to 24-fold compared to untreated control tumors in which only the conventional EPR effect is present [110]. This process is induced within a few minutes after NIR-PIT and is most marked in the first 6 h but becomes less pronounced afterward, reaching baseline levels by 24 h. Once nanodrugs have leaked into tumor beds, drugs can be retained there for several days before they eventually diffuse out. Dynamic fluorescence imaging and contrast-enhanced magnetic resonance imaging (MRI) can document the various types of nanosized agents, such as monoclonal antibody targeting

Figure 2 Cell killing mechanism of NIR-PIT.

(A) Cell killing mechanism of NIR-PIT. An antibody-IR700-antigen complex is formed on the cell membrane. With NIR light exposure, axial ligands are released from the IR700 molecule. The conformational change of the antibody-IR700-antigen complex may produce physical stress in the cell membrane, resulting in the weakening of the membrane. The water outside of the cell is flown into the cell to burst the cell. Adapted from Ref. [106]. (B) Single-molecule imaging of antibody-IR700 conjugates before and after NIR light exposure. A single molecule of pan-IR700 on mica changes in shape and size after NIR light irradiation. Adapted from Ref. [106]. (C) 3D LC-QPM imaging depicts morphological dynamics. 3T3/Her cells initially swelled, formed blebs, and ruptured after NIR light exposure. Red asterisk indicates a bleb. Yellow arrowhead indicates a flying fragment. Adapted from Ref. [107].

tumor, nontargeted PEG-coated quantum dots, iron oxide nanoparticles, and dendrimer-based nanosized contrast agents, which quickly accumulate in the tumor

(Figure 3A–C). Fluorescence histological study also shows the improved distribution of the APC within the deeper parts of tumors after NIR-PIT [112]. When NIR-PIT was

Figure 3 NIR-PIT induced SUPR effects.

(A) Schematic representation of the mechanism of the SUPR effect induced by NIR-PIT. After NIR light exposure, most of the initial cell killing occurs in the perivascular layer of tumor cells and a potential space is formed surrounding the tumor vasculature, resulting in increased vascular permeability and decreased interstitial pressures. As a result, enhanced nanodrug delivery can be observed. Taken from Ref. [111]. (B) Delivery of pegylated quantum dot 800 into an NIR-PIT-treated tumor increases up to 24-fold compared to a control tumor 1 h after pan-IR700-mediated PIT. Taken from Ref. [111]. (C) Super-enhanced delivery of gadofosveset, a protein-binding MRI contrast agent. Gadofosveset greatly and homogeneously enhances post-NIR-PIT-treated tumor. Circle indicates tumor. Adapted from Ref. [111].

combined with FDA-approved nanodrugs such as daunorubicine liposome or albumin-bound paclitaxel (Abraxane), therapeutic effects were dramatically enhanced compared to single therapy of either NIR-PIT or nanodrugs [110, 113].

5.3 Novel imaging technique for real-time imaging of the SUPR effect

Liang et al. monitored the hemodynamic changes in tumor vessels during PIT using novel imaging methods [114]. For

instance, optical coherence tomography (OCT) using light waves instead of sound waves has much better spatial resolution than ultrasound ($\sim 10\ \mu\text{m}$) [115]. Therefore, OCT can provide high-resolution 3D images of the tumor micro-architecture, including vessels and lymphatics [116]. OCT was used to obtain the structural information, Doppler OCT (DOCT) was used to measure the blood velocity, and speckle variance (SV) imaging was used to measure the vessel diameter [117–119]. DOCT imaging revealed that blood velocity in peripheral tumor vessels quickly dropped below the detection limit while the vessel lumen

remained open (Figure 4A). In contrast, histopathology immediately after NIR exposure showed the significant dilatation of tumor vasculature in the deep tumor bed. The peripheral blood velocity drop is due to the increase of blood pool volume within the treated tumor and implies a long drug circulation time, which can explain the hemodynamic basis of the SUPR effect.

Tang et al. examined the microdistribution of APCs during and after NIR-PIT using a minimally invasive two-channel fluorescence fiber imaging system and a high-resolution two-photon microscope with a microprism [120].

Figure 4 Hemodynamic changes in tumors induced by NIR-PIT.

(A) Hemodynamic changes in an NIR-PIT-treated tumor. (a–c) Representative OCT/DOCT/SV images at different time points (orange dashed lines) during PIT treatment. OCT provides the structure information. DOCT provides the blood velocity information and SV provides the size of the open lumen. (d) Time plot of the blood velocity (red line) and the vessel width (blue line). The blood velocity quickly drops below the detection limit with NIR light irradiation. The open space in the vessel lumen also decreases with time but remains open after 600 s of light irradiation. Adapted from Ref. [114]. (B) *In vivo* fluorescence intensity is measured by the two-channel fluorescence needle system. The normalized IR700 fluorescence intensity shows a greater drop in the tumor surface (PIT-s) and a higher recovery in deep tumor (PIT-d), although there was only a gradual decrease in controls (control-s and control-d). Adapted from Ref. [120].

The IR700 fluorescence intensity showed a quick drop both at the tumor surface and deep within the tumor during the NIR exposure, recovering quickly thereafter. The recovery of IR700 fluorescence intensity in deep tumor was significantly greater than that of the tumor surface and even reached higher level than the initial value of deep tumor (Figure 4B). Tang et al. also investigated the real-time microdistribution of APCs using OCT and high-sensitivity fluorescence laminar optical tomography (FLOT) [121]. FLOT is a 3D imaging approach that has a resolution of $\sim 100\ \mu\text{m}$ with 2–3 mm penetration depth [122, 123]. The 3D FLOT/OCT tumor images revealed that NIR-PIT could enhance the delivery of APCs especially into the areas that were initially difficult to reach and the recovered fluorescence intensity reached a higher level than the initial intensity due to SUPR effects.

6 Conclusion

Nanosized drugs have many attractive features for cancer chemotherapy because they can be highly loaded with anticancer agents and intrinsically result in preferable tumor delivery based on the EPR effect. However, the actual drug delivery based on the EPR effect is reduced by various factors, such as the leakiness of the tumor vasculature, high IFP, growth-induced solid stress, and solid stress from tumor stromal matrix. Even when the EPR effect can be amplified by pharmacological means or physical stimuli, the results are not consistent enough to substantially improve efficacy.

NIR-PIT is a newly developed cancer treatment that uses an antibody labeled with IR700 and kills only target cells without damaging nontarget cells. As NIR-PIT initially damages tumor cells adjacent to the tumor vasculature, there is a dramatic increase in permeability and retention immediately after treatment, which has been termed the SUPR effect. The SUPR effect improves nanodrug delivery up to 24-fold compared to control tumors. In addition, there is a possibility that the SUPR effect might enhance actively targeted nanodrugs. Thus, the SUPR effect could be a potent and practical strategy for nanodrug delivery to the tumor.

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