

Intratumoral Drug Delivery with Nanoparticulate Carriers

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ABSTRACT Stiff extracellular matrix, elevated interstitial fluid pressure, and the affinity for the tumor cells in the peripheral region of a solid tumor mass have long been recognized as significant barriers to diffusion of small-molecular-weight drugs and antibodies. However, their impacts on nanoparticle-based drug delivery have begun to receive due attention only recently. This article reviews biological features of many solid tumors that influence transport of drugs and nanoparticles and properties of nanoparticles relevant to their intratumoral transport, studied in various tumor models. We also discuss several experimental approaches employed to date for enhancement of intratumoral nanoparticle penetration. The impact of nanoparticle distribution on the effectiveness of chemotherapy remains to be investigated and should be considered in the design of new nanoparticulate drug carriers.

KEY WORDS extracellular matrix · intratumoral distribution · interstitial fluid pressure · nanoparticles · solid tumors

INTRODUCTION

Many solid tumors develop several biological features distinguished from those of normal tissues (1, 2). These features include defective blood vessels and the lack of

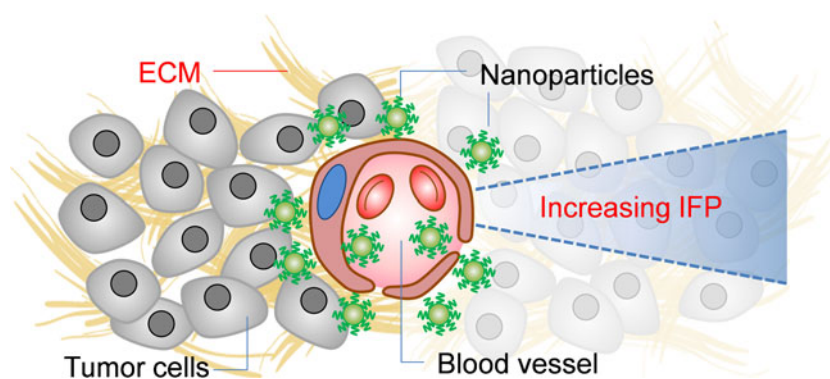
functional lymphatics (3), as well as abnormal interstitial properties such as increased stiffness of tumor extracellular matrix (ECM) (4) and relatively high interstitial fluid pressure (IFP) (5–11). In particular, the high IFP and abnormal ECM structure are known to be significant barriers to drug diffusion into the tumor mass. Ideally, an anti-cancer therapeutic should be able to reach tumors without systemic loss, penetrate all the way into the core of the tumor mass, enter tumor cells where their target molecules reside, and completely eradicate the tumors. However, the effect of an anti-cancer therapeutic is often limited to the periphery of the tumor mass close to the vasculature (12, 13). More centralized regions of the tumor remain unaffected (14), becoming a potential source for tumor relapse or metastasis. Several review articles discuss the challenges in intratumoral delivery of anti-cancer drugs (1, 11, 12).

In recent years, nanoparticles have been widely explored as a promising vehicle for delivery of anti-cancer therapeutics. The popularity of nanoparticles is attributable to size with a scale pertinent to cellular and subcellular functions and a surface that allows for multiple functionalizations (15). Submicron particles with appropriate surface protection can circulate for a prolonged period and accumulate in solid tumors via leaky vasculature, increasing drug delivery to tumors. On the other hand, due to the size of the system, nanoparticles are likely to face greater difficulties in penetrating tumors (Fig. 1) than free drugs. The effectiveness of nanoparticulate drug carriers may be fundamentally limited if they are designed without considering these physiological barriers (2). Despite the potential impact on clinical success of new delivery approaches, the tumor interstitium has not been actively investigated in the context of the nanoparticle-mediated chemotherapy. To enhance our understanding of tumor interstitium as an active player

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Fig. 1 A diagrammatic representation illustrating potential barriers to intratumoral transport of nanoparticles post-extravasation. ECM: extracellular matrix; IFP: interstitial fluid pressure.



in nanoparticle transport into solid tumors, thereby facilitating the development of effective nanomedicines, here we review the properties of tumor interstitium and discuss recent approaches to address consequential challenges in nanomedicine delivery.

STIFF TUMOR EXTRACELLULAR MATRIX

Composition and Functions of Extracellular Matrix (ECM)

Tissue stroma consists of fibroblasts, vascular pericytes, endothelial and immune cells, secreted growth factors, and ECM (16, 17). The ECM is composed of collagen networks, microfibril-elastin system (18, 19), glycosaminoglycan (GAG) such as hyaluronan, and proteoglycans (GAGs bound to protein) (18, 20). The ECM acts as structural support for neighboring vasculature and the basal membrane as well as a platform for cell attachment (18). Cell attachment to the ECM promotes intracellular signaling responsible for various cell activities such as cell cycle regulation, migration, and differentiation (21). Components of ECM have the ability to bind growth factors (20, 22) or drugs (6, 12), serving as a reservoir for the molecules.

As a dynamic entity, the ECM is involved in cell migration and remodeling of tissues in both healthy and diseased states (20). For example, proteoglycans in the tumor ECM communicate with growth factors or their receptors (23–27) and influence fibrosis and tumor growth (23, 28–32). Conversely, the ECM is remodeled according to the tumor progression (23, 33–35). Relatively stiff ECM is a common feature of many solid tumors. For example, high density of breast tissues has often been associated with tumorigenesis and metastasis in breast cancer patients (21, 36).

Causes of Stiff Tumor ECM

There are numerous reasons for the stiffness of tumor ECM, such as relatively high collagen levels, an increased

presence of lysyl oxidase (LOX), and enhanced integrin signaling in the tumor microenvironment.

High Collagen Levels

Collagen is produced by fibroblasts in the ECM and contributes significantly to the tensile strength of the tissues (37). Many solid tumor types exhibit stiff tumor ECM as a result of high collagen I levels (21). Studies have indicated that elevated levels of collagen I are an indicator of poor prognosis, metastasis and tumor reemergence (21, 38–40). Moreover, the high collagen levels in the tumor ECM are a major barrier in the transport of small-molecular-weight drugs, macromolecules, and nanoparticles (1, 33, 41).

Increased Presence of Lysyl Oxidase (LOX)

Copper-dependent lysyl oxidase (LOX) covalently cross-links collagen and, thus, stiffens the ECM (33, 42). LOX exists in a relatively high level in tumors (33, 43), induced by hypoxia inducible factor (HIF-1) and transforming growth factor beta (TGF- β) (33, 44). Relatively high expression of LOX has been linked to metastasis in squamous carcinomas (21, 45) and poor prognosis. Inhibition of LOX reduces collagen crosslinking as well as LOX-mediated focal adhesions that lead to mammary epithelial cell invasion (33).

Enhanced Integrin Signaling

Integrins are cell receptors that allow communication between cells and the ECM (21). Integrins bound to components of the ECM influence cell survival through signaling, while unbound integrins signal a need for apoptosis (46–48). Different integrins are expressed in various tumor types. For example, the $\alpha\beta3$ integrin is expressed in melanoma and glioblastoma as well as tumors of the breast, prostate, pancreas, ovary, and cervix (46). It is suggested that ECM stiffness enhances integrin expression, and integrins subsequently promote focal adhesions of cells

(4, 33, 49), promoting cytoskeleton remodeling (33, 50). Additionally, integrins can directly influence tumor cell growth (46, 51, 52).

Influence of Stiff Tumor ECM on Drug Transport

Transports of immunoglobulin G (IgG, 150 kDa) and bovine serum albumin (BSA, 68 kDa) in the tumor ECM were compared in a variety of tumor xenograft models, such as human colon adenocarcinoma (LS174T), human glioblastoma (U87), human soft tissue sarcoma (HSTS 26T), and murine mammary carcinoma (MCAIV), which have different elastic moduli (41). BSA diffusion in the tumor ECM was moderately hindered as compared to that in buffered saline, but there was no significant difference according to the tumor models. On the other hand, IgG diffusion was slowed to a greater extent in relatively stiff tumors (41). By comparing the compositions and structures of the tumors, the authors concluded that the resistance to macromolecule penetration was due to the difference in collagen organization rather than the hyaluronan or total GAG content, suggesting that it is the collagen network that should primarily be addressed for efficient intratumoral delivery of macromolecules (41). Here, one should note that the hydrodynamic radii of BSA and IgG are approximately 4.5 nm (53) and 5.3 nm (54), respectively, at least one or two orders of magnitude smaller than those of nanoparticles used for biomedical applications (gold nanoparticles (10–100 nm) (55–57), liposomes (100–1,000 nm) (58–60), or polymeric drug delivery vehicles (50–300 nm) (61–63)), yet their movements in the tumor ECM were significantly hindered by the collagen network.

HIGH TUMOR INTERSTITIAL FLUID PRESSURE

Tumor Interstitial Fluid Pressure (IFP)

Interstitial fluid is similar to plasma in the electrolyte composition and contains approximately 50–60% of the proteins present in the plasma (18). The composition of interstitial fluid depends on the size and charge of substances that diffuse from the blood across capillary walls (18, 64). Traditionally it has been thought that the interstitial fluid is a reservoir for the extra fluids, and its volume is passively controlled in response to the perturbations of capillary filtration and lymph flow (65). However, a recent view suggests that IFP is actively controlled by tension on the collagen network exerted by fibroblasts, a process mediated by various growth factors like platelet-derived growth factor (PDGF) (66, 67) and transforming growth factor- β (TGF- β) (68).

Relatively high IFP is a common feature of many solid tumors (11, 69, 70). Normal IFP is typically between -3

and 3 mmHg, but tumor IFP is significantly higher than normal, ranging from 5 to 40 mmHg and even reaching 100 mmHg (71–75). Hofmann *et al.* determined that IFP increases with increasing tumor volume (75–78). The degree of elevated IFP in the tumor is linked with poor prognosis (72, 79, 80).

Causes of High Tumor IFP

High tumor IFP is attributable to the relatively high permeability of the vasculature, increased contractility of stroma cells, and lack of functional lymphatic system (1, 71, 73, 75, 81, 82). Rapidly growing tumors recruit new blood vessels via secretion of growth factors like vascular endothelial growth factor (VEGF) (83–85), PDGF (86, 87), and TGF- β as well as other angiogenic factors (11). Due to the lack of elaborated control of angiogenic processes, tumor vasculature is typically irregular and convoluted and lacks regular pericyte coverage, accounting for the leakiness of the blood vessels (11). Fibroblasts of tumor stroma gain contractile function by differentiating towards smooth-muscle cells (88) and exert increasing pressure on the ECM (11). Moreover, many tumors do not have normal lymph vessels, which are responsible for returning macromolecular solutes and interstitial fluids back to the bloodstream (1). The lack of functional lymph flow results in inefficient removal of solutes and fluids from the tumor interstitium, further increasing the IFP (11).

Influence of High Tumor IFP on Tumor Progression

IFP influences tumor metastasis, responses to radiation treatment, and patient survival, although the biological mechanisms remain unclear (89). Rofstad *et al.* reported that high IFP promotes pulmonary and lymph node metastasis of A-07 tumors (90). This group also observed in a large A-07 melanoma xenograft model that tumors with high IFP had high fractions of acutely hypoxic cells and were resistant to radiation treatment (89). In a subsequent study with small A-07 and R-18 melanoma xenografts without hypoxia, they observed that tumors with high IFP were relatively less sensitive to radiation therapy, indicating that the high IFP negatively influenced the radiocurability in a hypoxia-independent manner as well (71). High IFP also stimulates tumor cell proliferation by exerting the mechanical forces on the cells (91, 92).

Influence of High Tumor IFP on Drug Transport

Tumor IFP is a significant physiological barrier in the delivery of therapeutics to the tumor site, resulting in uneven drug distribution within the tumor mass (1, 93). The difficulty increases with the size of a therapeutic

molecule, which is transported by convection rather than a concentration gradient (diffusion) (94, 95). The high IFP induces fluid flow in an undesirable direction—from the high-pressure core to the tumor periphery, preventing effective penetration of macromolecular therapeutics (94).

DISTRIBUTION OF NANOPARTICLES IN TUMOR MASS

With the recent advances in imaging techniques, a number of studies have demonstrated biodistribution of nanoparticles in animal models (96–99). Irrespective of the presence of a cell-specific ligand on the surface, nanoparticles tend to accumulate in the solid tumors via the leaky vasculature and the impaired lymphatic drainage, as long as they can circulate for a prolonged period (100–102).

On the other hand, the post-extravasation fate of nanoparticles varies with particle properties, including size, surface charge, and affinity for the cells. For example, Zhang *et al.* found that penetration of transferrin receptor-targeted lipopolyplexes into three-dimensional cell clusters was relatively limited as compared to a free payload (antisense oligonucleotide) (103). Consequently, the targeted lipopolyplexes were less effective in down-regulating the target gene (Bcl-2) expression than free oligonucleotides *in vivo*, despite the greater amount deposited in the tumors (103). This result may first be attributed to the large size of lipopolyplexes (140 nm *vs.* 18-mer oligonucleotide), but the contribution of surface charge (positive charge for lipopolyplexes) and affinity for the cells (due to the transferrin-mediated interaction) cannot be ignored.

Particle Size

An ideal nanoparticle size for tumor accumulation via the leaky vasculature is considered to be in the range of 10–100 nm, above the threshold for the renal filtration, although the upper limit is not well defined (15). In earlier studies, particles even at the upper end of this range were thought to be able to penetrate the tumors. For example, Nomura *et al.* observed that 85 nm emulsion and 120 nm liposomes appeared immediately in the venous outflow after intratumoral injection (104). In addition, Reddy *et al.* reported that polymeric nanoparticles with an average size of 178 nm penetrated through the tumor interstitium after peritumoral injection (105). In another example, 65 nm polymeric micelles penetrated a multicellular spheroid (106). The micelles released doxorubicin in the cells over 3–24 h, unlike free doxorubicin that appeared in the cells in <1 h, implying that extracellular drug release was minimal and intact micelles penetrated the spheroid until they reached the cells (106). However, it is uncertain whether it

was indeed the intact nanoparticles of those sizes that traveled through the tumor matrix, since these studies were based on observation of particle components or payloads, which may not necessarily represent the nanoparticle assemblies.

Recent studies suggest that the size threshold appropriate for tumor penetration would be much lower than expected. Based on a mathematical model, Goodman *et al.* predicted that polystyrene particles of 20–40 nm would be able to accumulate in the interior of a multicellular spheroid, but 100 or 200 nm particles would not (107). A similar size limit was experimentally observed in a recent study using a mouse model of human breast cancer (108). This study reported that the intratumoral distribution of 25 nm polymeric micelles was relatively broad. In contrast, 60 nm micelles were localized in proximity to the blood vessel (108).

Surface Charge of Particles

Neither cationic nor anionic particles are desirable for long-term circulation, due to the propensity to recruit opsonins and stimulate phagocytosis among many other effects (109–111). On the other hand, cationic surface charge increases particle binding to the vessels via electrostatic interactions with the vascular glycocalyx (112, 113). Combined with sluggish and irregular tumor blood flow, this feature is expected to contribute to targeted drug delivery to tumoral vasculature (112).

On the other hand, Campbell *et al.* observed that cationic liposomes (150 nm) did not travel far into the tumor interstitium (112). The effect of surface charge on nanoparticle penetration through the tumor interstitium was more systematically investigated in recent studies. Kim *et al.* compared the penetration of oppositely charged gold nanoparticles (+30 *vs.* –36 mV, 6 nm) into cylindrical cell aggregates (114). Cationic nanoparticles were taken up by the proliferating cells on the periphery of the cylinders, releasing drug in the cells, whereas anionic nanoparticles were better at penetrating the extracellular matrix and entered hypoxic, necrotic cells in the core of the mass (114). The authors also compared diffusivity of the gold nanoparticles in Matrigel, a tumor extracellular matrix material (114). Cationic nanoparticles had a diffusion coefficient significantly lower than that of anionic particles, due to the attractive electrostatic interactions with negatively charged proteoglycans (114). Stylianopoulos *et al.* also showed both in modeling and in experimentation that quantum dots with a positive charge (33.1 mV, 5.8 nm) had a lower diffusion coefficient than PEGylated quantum dots (–11.5 mV, 5.6 nm) in a collagen gel (115).

Not only cationic particles but anionic particles are also likely to be limited in intratumoral movement (116). Lieleg *et al.* observed that the mobility of highly charged particles

(160–170 nm), either negative or positive, was significantly reduced compared to PEGylated ones, even though the particles were sufficiently smaller than the mesh size (2–3 μm) of an ECM hydrogel (116). The limited mobility of particles in the ECM was attributed to electrostatic attractive interactions between the particles and the ECM biopolymer network, which had both cationic and anionic patches (116). Addition of agents that could shield the surface charge of particles improved the particle movement (116). Recently, Stylianopoulos *et al.* discussed that the hindrance in intra-ECM movement of highly charged particles was also due to repulsive interactions with biomacromolecule fibers, especially when the fibers were relatively thin (115). According to these studies, neutral particles would be the most desirable for transport into the tumor matrix (115, 116).

Tumor-Targeting Ligands

The presence of cell-specific ligands influences the distribution and retention of nanoparticles in the tumor mass. Kirpotin *et al.* reported that HER2-targeted immunoliposomes accumulated within tumor cells, whereas non-targeted liposomes were located predominantly in the ECM (101). Epidermal growth factor receptor (EGFR)-targeted immunoliposomes were found inside the tumor cells six times more than non-targeted liposomes (117). In another example, folate receptor (FR)-targeted liposomes were injected intravenously to mice with ascitic lymphoma to examine the distribution of liposomes (118). The overall accumulation of FR-targeted liposomes in ascites was somewhat lower than that of the non-targeted ones, but the fraction of FR-targeted liposomes associated with tumor cells was much higher compared to non-targeted liposomes (118). These studies show that the presence of cell-specific ligands enhances the retention of nanoparticles in the tumor mass via interactions with tumor cells. Farokhzad *et al.* compared the anti-tumor effects of intratumorally injected nanoparticles and found that non-targeted particles were inferior to targeted ones (119). The difference in the anti-tumor effect between the two particles, given in the same dose directly to the tumors, is attributable to the cell-nanoparticle interaction that would enhance the retention of nanoparticles in the tumors (119).

On the other hand, the cell-specific ligands can interfere with penetration of nanoparticles to the tumor mass. Jang *et al.* discussed that the spatial distribution of a drug in tumor spheroids depended on the affinity of the drug for the cell membrane (1). While molecules that do not bind to cell membrane readily penetrate the spheroids, those binding to the cellular macromolecules remained at the periphery of the spheroids (1). In earlier studies, Weinstein *et al.* pointed that intratumoral distribution of cell-specific

antibodies is heterogeneous due to the “binding site barrier,” a phenomenon whereby high-affinity antibodies travel a limited depth from the tumor surface (120, 121). The binding site effect was observed with EGFR-targeted micelles in a recent study (108). Lee *et al.* compared the intratumoral distribution of targeted micelles and non-targeted micelles using confocal microscopy of sectioned tissues and found that the mean diffusion distance of targeted micelles from the blood vessels was significantly shorter than non-targeted ones (108).

APPROACHES TO ENHANCE INTRATUMORAL NANOPARTICLE TRANSPORT

Enzymes Degrading the ECM

Reducing the stiffness of the ECM and IFP should enhance the permeation of nanoparticulate drug delivery systems through the ECM and enable more homogeneous drug distribution across the tumor mass. Additionally, given that the stiff ECM is associated with tumorigenesis (33, 122), weakening the matrix may interfere with some of the cell-cell and cell-matrix interactions that promote tumor growth. Collagenase (123, 124) and hyaluronidase (123, 125) have been used in conjunction with drug delivery systems to study how these enzymes influence drug distribution in tumors. Moreover, Barkan and others have suggested that hindering the buildup of collagen I as well as LOX-mediated collagen crosslinking may impede tumor reemergence (21).

A few studies reported that hyaluronidase treatment sensitized drug-resistant tumors to chemotherapy, presumably by facilitating drug diffusion into the tumors (126–128). However, Netti *et al.* suggested that this effect might be due to other mechanisms than enhanced ECM permeability, given the lack of relationship between hyaluronan content and interstitial mobility of macromolecules (41). These authors reported that collagenase treatment improved the penetration of IgG in tumors with rigid ECM and suggested that collagen would be a more reasonable target (41). Additionally, this group showed that chronic treatment of relaxin, a hormone up-regulating collagenase production, induced degradation of collagen and enhanced transport of macromolecules like IgG and 2 MDa dextran into the tumors (129, 130).

The ECM-degrading enzymes have been used to promote intratumoral nanoparticle transport. Hyaluronidase was used to improve the uptake of liposomes in tumors. Liposomal doxorubicin (Caelyx™) was injected intravenously into human osteosarcoma xenografts (subcutaneous or orthotopic) 1 h after intratumoral or intravenous injection of hyaluronidase (125). Intratumoral injection of

1,500 U hyaluronidase reduced IFP by ~40% in orthotopic xenografts (125). Intravenous injection of hyaluronidase in a subcutaneous xenograft model showed a similar decrease in IFP but required a longer time than intratumoral injection for maximum response (125). Notably, the hyaluronidase treatment enhanced the tumoral distribution of liposomal doxorubicin. Doxorubicin distribution was heterogeneous in tumors of untreated animals. With hyaluronidase, doxorubicin was found in the periphery as well as in the central part of the tumor (125). Hyaluronidase did not influence the microvascular pressure or the intracellular localization of doxorubicin in nuclei, indicating that its main function was to reduce IFP, presumably by degradation of ECM (125).

Goodman *et al.* used a mathematical model to predict transport of polystyrene nanoparticles (20–200 nm) through spheroids and verified with *in vitro* experiments. Multicellular spheroids of human cervical carcinoma (SiHa) cells were grown to 400–500 microns for simulation of avascular regions of solid tumors (107). Fluorescent polystyrene nanoparticles were introduced from the periphery of the spheroids and optionally treated with collagenase, and the fluorescence level was observed across the section of the spheroids (107). The authors observed pronounced cell loosening and shedding at the periphery of the spheroids and increasing porosity in the middle quiescent region due to the collagenase treatment (107). Without collagen treatment, the nanoparticle distribution was limited to the periphery of the spheroids, irrespective of particle size. On the other hand, diffusion of 20 or 40-nm nanoparticles was significantly enhanced by the collagen treatment at the periphery of the spheroid as well as toward the necrotic center with a higher porosity (107). However, collagen treatment was ineffective for larger nanoparticles (100 nm or 200 nm) (107).

Kuhn *et al.* created proteolytic superparamagnetic (SPM) nanoparticles (145 nm in radius), whose surface was modified with collagenase. Penetration of proteolytic SPM nanoparticles was observed in an enhanced ECM matrix, supplemented with additional collagen (131). Under the influence of external magnetic field, the collagenase-conjugated SPM nanoparticles were capable of penetrating the ECM at a rate of ~90 $\mu\text{m}/\text{h}$ (131). The mobility of collagenase-conjugated SPM particles was proportional to the number of functional collagenases on the NPs (131). In contrast, albumin-conjugated SPM nanoparticles were unable to penetrate the enhanced ECM (~0 $\mu\text{m}/\text{h}$) (131). Similarly, penetration of collagenase-coated polystyrene nanoparticles (100 nm) was four times higher than albumin-coated particles, according to the number of particles delivered to the core of the tumor cell spheroid (132).

Lowering Tumor IFP

Several studies show that reduction of IFP increased transport of therapeutics into tumor masses (11). A representative approach is to normalize the tumor vasculature by inhibiting angiogenic growth factors to an optimal level (133, 134). Jain suggested that inhibitors of the angiogenic growth factors exert an effect on cancer therapy as an adjuvant to chemotherapy rather than an active killer of tumor vasculature as originally envisioned (134). In this context, the role of anti-angiogenic factors was to normalize the abnormal tumor vasculature during the course of therapy, thereby facilitating oxygen and drug delivery (134). One of the desirable effects of anti-angiogenic agents would be to lower tumor IFP. Anti-VEGF monoclonal antibody reduced IFP by more than 70% in human rectal tumors (9) as well as xenograft mouse models of human glioblastoma multiforme (U87) and colon adenocarcinoma (LS174T) (73). Similarly, DC101, a VEGF-receptor-2 antibody, decreased the IFP and induced morphological and functional changes in the vascular network, facilitating BSA penetration in tumors (135).

Alternatively, PDGF antagonists were used to reduce contractility of fibroblasts in the tumor ECM. Imatinib (ST1571), a tyrosine kinase inhibitor of PDGF receptors, was administered concomitantly with epothilone B, a microtubule stabilizer (136, 137), to treat a mouse model of human anaplastic thyroid carcinomas (86). The imatinib treatment decreased tumor IFP, increased the tumor levels of epothilone B by three-fold, and enhanced anti-tumor effects of epothilone B (86). Similar effects were observed with a combination of inhibitory PDGF aptamer or imatinib with Taxol in rodent tumor models (79, 138). However, the effect of imatinib on IFP and drug uptake was transient, and the IFP returned to the pre-treatment level once the imatinib treatment ended (86).

Recently, Klosowska-Wardega *et al.* tested a combination of anti-PDGF and anti-VEGF therapies to augment the response to Taxol-based chemotherapy (83). This combination additively reduced the IFP of KAT-4 tumors in severe combined immunodeficient (SCID) mice, but it did not translate to further enhancement of anti-tumor efficacy of Taxol, likely due to the suboptimal dosing schedule (83).

Additional agents explored to decrease tumor IFP and increase drug uptake are reviewed elsewhere (11). These agents include nicotinamide (139), dexamethasone (140), tumor necrosis factor-alpha (TNF- α) (141–143), Prostaglandin E₁ (PGE₁) (144, 145), bradykinin agonist (146), TGF- β inhibitor (147), and hyaluronidase (148). However, due to the side effects, systemic application of some agents is cautioned (75, 125, 142).

Hofmann *et al.* infused 20% human serum albumin (HSA, 66 kDa) in a xenograft model to increase the plasma colloid osmotic pressure so that fluid diffused from the interstitium back into blood vessels (75). The administration of 20% HSA reduced tumor IFP from 8 to 2 mmHg and increased the uptake of dextran (40 kDa) and cetuximab (151.8 kDa) by tumors (75). The tumor growth was better inhibited by the combined administration of 20% HSA and cetuximab than cetuximab alone (75). Dextran was taken up in the tumor mass relatively quickly but flushed from the tumor mass quickly as well (75). On the other hand, cetuximab was taken up by the tumor mass relatively slowly but remained in the tumor tissue due to the affinity for the A431 carcinoma cells (75).

Priming Tumors with an Apoptosis-Inducer

Drug penetration into the tumor mass can be enhanced by expanding the interstitial space of tumors using an apoptosis inducer. Au, Wientjes, and colleagues observed that paclitaxel penetration into three-dimensional tumor histoculture increased abruptly 12–24 h after the treatment (149, 150). They suggested that the enhanced drug penetration was related to the decreasing cell density resulting from drug-induced apoptosis (149). In a subsequent study, they confirmed that paclitaxel accumulation and tumor apoptosis were significantly higher when the tumors were pre-treated with a small dose of paclitaxel (“primed”) prior to the second dose than when the same total dose was administered at once (151). Recently, this group reported a microparticle system that released paclitaxel at different rates so that the first dose of paclitaxel was rapidly released to prime the tumors, followed by the second that provided the sustained drug release (152). This system achieved greater drug level in ovarian tumors, lower toxicity, and longer survival than the commercial Taxol formulation in an ovarian SKOV-3 xenograft model (152). In addition to decreasing tumor cell density, paclitaxel has an effect on tumor vessels (153). Griffon-Etienne *et al.* observed that paclitaxel increased the diameter of tumor vessels and decreased the microvascular pressure and IFP (153), which may be an additional mechanism of the priming effect of paclitaxel.

Application of Magnetic Force

Kuhn *et al.* studied magnetic force-induced penetration of SPM nanoparticles through purified ECM (154). The SPM nanoparticles had either a silica coating (135 nm in radius) or a 300 Da polyethylene glycol (PEG) coating (145 nm or 400 nm in radius) (154). The ECM was isolated from Engelbreth-Holm-Swarm murine sarcoma and allowed to

polymerize before nanoparticles were applied to the gel surface (154). In the absence of an external magnetic field, none of the nanoparticles penetrated the polymerized ECM (154). When the magnetic field was applied, only 145-nm PEG-coated nanoparticles could travel through the ECM at a velocity of 1.5 mm/h, whereas 400-nm PEG-coated nanoparticles and 135-nm silica-coated particles, which aggregated in the experimental condition, were virtually immobile (154).

Combination with Radiofrequency (RF) Thermal Ablation Therapy

Focal solid tumors can be treated with RF thermal ablation, an induction of coagulation necrosis of tumors using brief heating (<15 min) at >50°C via needle electrodes (155). Goldberg *et al.* observed a synergistic effect between intratumoral doxorubicin injection and RF ablation in destruction of solid tumors in a rat model (156). In a subsequent study, this group used intravenous application of doxorubicin liposomes (Doxil) in conjunction with RF ablation and achieved higher tumor destruction than Doxil and RF therapy alone (157). The authors attributed this synergistic effect in part to enhanced accumulation of Doxil due to the increased vascular permeability and blood flow (155). Indeed, autoradiographic studies using radiolabelled liposomes demonstrated high liposome distribution in the area peripheral to the RF-ablated region (158). Recently, Zhang *et al.* predicted, using a mathematical model, that liposome penetration into the avascular central region of tumors would still be limited, but released free drug would diffuse into the central region, which has already been destroyed by RF ablation, and eradicate remaining cells (159). In this context, the authors proposed the use of RF ablation in conjunction with temperature-sensitive liposomes that release drugs by heating (159).

FUTURE PERSPECTIVES

Studies have shown in animal models that nanoparticles that circulate for a prolonged period can increase the distribution of the encapsulated drug in solid tumors with leaky vasculature (160). On the other hand, intratumoral distribution of nanoparticles post-extravasation remains relatively unexplored, with a limited number of studies performed *in vitro*, such as in multicell spheroid models or collagen matrices. Recently, Lee *et al.* have demonstrated *in vivo* that the distribution of polymeric micelles in the tumor mass was mainly localized in regions with higher blood vessel densities (108, 161). The heterogeneity of intratumoral nanoparticle distribution would be attributable to

biological features of solid tumors that resist intratumoral penetration of nanoparticles, such as the stiff ECM structure, elevated IFP, and affinity for the tumor cells in the peripheral region of a solid tumor, which have long been recognized as diffusion barriers to small-molecular-weight drugs and antibodies (1, 11, 12). Particle size, surface charge, and the presence of cell-specific ligands are found to play critical roles in nanoparticle distribution in tumors. Although it remains to be investigated, particle shape (162) or flexibility (163) may also have an impact on the intratumoral nanoparticle transport.

It is yet unclear whether homogeneous intratumoral distribution of nanoparticles (if it is possible) would be superior to localized distribution of a large amount of nanoparticles to the region proximal to blood vessels. Intratumoral particle penetration may be more desirable as it overcomes the consequences of heterogeneous blood flow in tumors (2) and provides a broad coverage. Alternatively, concentrating nanoparticles in the periphery of tumor may be a more effective way of killing actively proliferating tumor cells in that region. With recent advances in nanotechnology and imaging techniques, we may be able to find answers to these questions: how different attributes of nanoparticles influence their intratumoral distribution and, consequently, the effectiveness of nanoparticle-mediated chemotherapy. It is high time to initiate a systematic exploration of this important yet relatively under-explored area, and it should be an integral part of our effort to build new nanoparticulate drug carriers.

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