Review Article
Strategies to improve drug distribution in solid tumor

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Abstract: Most research on the resistance of cancers to chemotherapy has concentrated on molecular mechanisms of resistance, whereas the role of limited drug distribution within tumors has been neglected. One critical obstacle to the success of anticancer therapies, especially to chemotherapy, radiotherapy and immunotherapy, is related to the inefficient distribution of drugs or oxygen to cancer cells. To be most effective anticancer drugs must penetrate tissue efficiently, reaching all the cancer cells that comprise the target population in a concentration sufficient to exert a therapeutic effect. The causes of the inefficient distribution of the anticancer compounds in the tumor bulk are multiple and interconnected. Obviously, the penetration capacity of a drug depends on its physicochemical properties, but the reasons for low delivery can be mainly ascribed to the tumor microenvironment, such as absent or immature vessels, irregular blood flow, high interstitial fluid pressure (IFP), low oxygen (hypoxia) and high acidity. In this article, we discuss the potential strategies to improve the drug distribution by modifying factors such as tumor vessels, tumor blood flow, tumor stroma, tumor cells, interstitial fluid pressure (IFP), and drug properties.

Keywords: Drug distribution, tumor microenvironment, antiangiogenesis, proangiogenesis, anticoagulation

Introduction
Beyond the difficulty of delivering drugs through chaotic tumor vasculature and the dangerous fluid buildup caused by leaky vascular walls, the abnormalities of tumor vessels create a highly unnatural microenvironment inside a tumor as well. Because many areas of a tumor lack vasculature and existing vessels are unable to deliver sufficient oxygen to surrounding tissues, a general state of hypoxia (low oxygen) and high acidity prevails in the tumor. The body’s immune cells, which might help fight a tumor, are hampered by acidity and cannot function in low oxygen. Nor can radiation treatments and a subset of chemotherapy drugs that depend on chemical processes that require oxygen to kill cancer cells [1-3]. In addition, the disorganized vascular network and the absence of functional lymphatics [4, 5] causes increase interstitial fluid pressure (IFP) [6-8]. Overall, these characteristics of the tumor microenvironment limit the delivery of anticancer drugs to cells that are situated distal from functioning blood vessels.

In this article, we review the current state of knowledge of the potential strategies to overcome the obstacles of impeding the penetration or distribution of anticancer agents into human solid tumors.

Vascular normalization of antiangiogenic therapy
The seminal work by Folkman on tumor angiogenesis stimulated the discovery and development of many angiogenesis inhibitors [9]. The most validated antiangiogenic strategies act on the VEGF axis, blocking VEGF directly with the neutralizing antibody bevacizumab or the VEGF trap, or indirectly with low-molecular-weight tyrosine kinase VEGF receptor inhibitors (e.g., sunitinib, sorafenib, and pazopanib) [10]. In terms of drug delivery, one would expect that the antiangiogenic treatment, by altering tumor vasculature, impairs the delivery of chemotherapy. The fact that the antiangiogenic drugs enhance the response to anticancer drugs when given in combination suggests that they do not necessarily decrease drug delivery to
Improving drug distribution in solid tumor tissue. Jain [11] has proposed that antiangiogenic drugs induce a process of vascular normalization. It is a transient reversion of the irregular tumor vasculature to a normal state, with a consequent drop in IFP and reduction of hypoxia, that provides an improvement of the penetration and activity of concurrent cytotoxic agents.

Wildiers et al. [12] showed that the administration of an anti-VEGF monoclonal antibody to mice bearing a colon adenocarcinoma at 1 week before irinotecan administration causes a higher tumor perfusion and an increase in the intratumor irinotecan concentration. Dickson et al. [13] reported that the treatment of orthotopic neuroblastoma xenografts with bevacizumab results in a sustained decrease in both tumor vessel permeability and IFP, with a concomitant increase in intratumor perfusion for 1 week. The penetration of topotecan and etoposide improved when given at 1-3 days after bevacizumab as compared with concomitant administration or with a dosing schedule with a 7-day interval [13]. These findings indicate that the effect of the antiangiogenic therapy is transient, generating a narrow window of time during which synergy can be achieved. Consequently, it is of the utmost importance to carefully define the timing of the normalization window, the scheduling, and the dosing of antiangiogenic therapies in order to optimize the efficacy of a combination of antitumor strategies.

Vascular normalization, using antiangiogenic agents, is the process by which partial loss of blood vessel density is associated with a temporary increase in blood flow [14]. This approach has shown significant promise [15, 16], but since it relies on a temporal window of opportunity that is both time and dose dependent and may well be different for different cancer types, it is generally considered difficult to implement clinically [17]. The available clinical information on the influence of antiangiogenic drugs on the distribution of anticancer drugs given in combination is limited. Willet et al. [18] performed a study in six patients with rectal adenocarcinoma showing that bevacizumab at the dose of 5 mg/kg in combination with 5-fluorouracil and radiotherapy is able to reduce the IFP from 15 to 4 mmHg. This effect was associated with decreased tumor blood perfusion and vessel density.

Proangiogenesis: vascular improving therapy

Although the non-uniform coverage does not allow the same sophisticated mode of regulation as that of coronary arteries, tumor blood vessels have been shown to respond to a variety of substances [19]. Vasoactive drugs able to selectively increase tumor blood flow have the potential to act as radio- and/or chemosensitizers. Although many drugs tested so far do not meet this last requirement, they were used in early studies to test whether modulation of the functional vascular reactivity within tumors is at least a feasible objective.

Bradykinin and angiotensin II are well-known physiological peptides exerting opposite vaso-dilatory and vasoconstricting effects, respectively. Amazingly, they have both been documented to improve drug delivery in tumors. Labaradimil, a bradykinin agonist, was shown to increase the delivery of carboplatin through a decrease in IFP [20-22]. Whether the tumor vasculature contained a higher density of Beta-2 receptors in these studies is not known; however for maximal efficacy, the bradykinin agonist had to be administered at low dosage and after the chemotherapeutic drug infusion [20, 21].

Calcium antagonists were historically among the first agents evaluated for their effects on the tumor vasculature. Nifedipine application was recently shown to enhance tumor microcirculation [23]. Other investigators also focused on another vasomodulatory molecule, endothelin-1 (ET-1). ET-1 is known as powerful vasoconstrictor molecule even though, when delivered intravenously, the normal vasculature first responds by a transient vasodilation. Several reports have described the effects of ET-1 antagonists on drug and oxygen delivery to the tumor [24, 25]. Administration of ETA antagonists to tumor-bearing mice was reported to lead to a significant increase in tumor blood flow and oxygenation [25]. Another study also reported that these effects could contribute to a better delivery of cyclophosphamide into the tumor and a subsequent significant tumor growth delay [24].

Nicotinamide is another example of a drug thought to influence tumor vessel function through an effect on heterogeneities in tumor perfusion. Its proposed action on tumor oxy-
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Radiosensitization is clinically exploited to radiosensitize tumors [26] and a few experimental studies have also documented its use as adjuvant for chemotherapy [27]. Another possible method for improving drug delivery is to modulate the muscle tone of blood vessels with, for example, the use of histamine [28] or a selective endothelin receptor A antagonist [25, 29], which would increase tumor blood flow. Botulinum neurotoxin type A induces relaxation of tumor vessels and has been shown to promote in vivo tumor perfusion and to delay tumor growth when combined with cyclophosphamide [30].

By contrast to anti-angiogenic strategies, Feron et al. have proposed the term “provascular” for any attempt to temporarily increase tumor perfusion/oxygenation through pharmacological/physical interventions [19]. Wong et al. [31] have proposed an alternative approach called “vascular promotion therapy”. Low doses of the antiangiogenic drug cilengitide can enhance tumor angiogenesis, and the calcium channel blocker verapamil can increase vessel dilation and blood flow; therefore, the authors hypothesized that these two agents would improve delivery of the chemotherapeutic agent gemcitabine when administered in combination. Analysis of the tumor vasculature showed that the triple combination of cilengitide-verapamil-gemcitabine increased blood vessel density and perfusion compared with treatment with gemcitabine alone. Further experiments demonstrated that the combination treatment increased vessel leakage and reduced tumor hypoxia, and enhanced intratumoral drug delivery and thus the overall efficacy and potency of gemcitabine. This study provided an interesting approach to cancer treatment by promoting, rather than inhibiting, vascular formation. These results are unexpected and call for consideration of vascular promotion strategies in combination with chemotherapy for the treatment of cancer. These results point toward a possible radical change in therapeutic strategy by vascular promotion which allows significantly reduced doses of chemotherapeutics to be used effectively. By enhancing intratumoral delivery and intracellular uptake of the cytotoxic drug, vascular promotion therapy can minimize adverse effects of the therapy, while enhancing its efficacy. Thus, this strategy could provide the opportunity to extend treatment duration without reducing quality of life.

Anticoagulation for improving drug distribution in tumor

Up to 50% of all cancer patients and 90% of those with metastatic disease have coagulation abnormalities [32]. A hypercoagulable or prothrombotic state of malignancy occurs due to the ability of tumor cells to activate the coagulation system. It has been estimated that hypercoagulation accounts for a significant percentage of mortality and morbidity in cancer patients. There is considerable evidence that thrombosis is a common complication of malignancy, and represents the second most frequent cause of death in cancer patients [33, 34]. A broad spectrum of clinically significant hemostatic abnormalities may afflict as many as 15-25% of cancer patients. Furthermore, hemostatic complications are the second most common cause of mortality in cancer patients, particularly in those with pancreatic, gastrointestinal or lung cancer, and 10% of newly diagnosed myeloma patients treated with any type of chemotherapy develop deep venous thrombosis [34-36].

The prothrombotic state in cancer patients originates from the highly abnormal hemodynamic system in tumors with direct interactions between cancer cells and endothelial cells, platelets, or monocytes. There is also an imbalance in procoagulatory and fibrinolytic (anticoagulatory) factors induced by the tumor. Endothelial cells, macrophages, and cancer cells in tumors express tissue factor and cancer procoagulant, which activates the coagulation cascade [32, 34, 37]. Although vascular normalization of antiangiogenic agents can transiently remodel tumor vessels and partially overcome the physiological barriers to drug and oxygen delivery within tumors and vascular promotion therapy can increase chemotherapy delivery and intracellular uptake of the drug, these strategies cannot reverse the hypercoagulable or prothrombotic state of malignancy and restrict their synergic efficacy when given in combination with chemotherapy. Improving the hypercoagulable or prothrombotic state of malignancy to reinforce the antitumor efficacy of vascular normalization or vascular promotion therapy in combination with chemotherapy is highly desirable. With these issues in mind, researchers began to explore the potential application of anticoagulation as adjuvant ther-
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apy for treatment of cancer. Aspirin, for example, several important observational studies published in the past 3 years strongly indicate that aspirin treatment after (or before) the diagnosis of colorectal cancer reduces distant metastasis and improves colorectal cancer-specific mortality [38, 39].

Herein, we hypothesize that anticoagulation therapy in combination with vascular normalization of antiangiogenic agents or vascular promotion therapy and chemotherapeutics could result in a synergic antitumor efficacy. This hypothesis based on the following facts: Firstly, anticoagulation therapy can improve the hypercoagulable state of tumors, increase the penetration capacity of chemotherapeutics, and improve the efficient distribution of chemotherapeutics to cancer cells, which result in enhanced antitumor efficacy of chemotherapy. Furthermore, anticoagulation therapy can improve the microvascular environment to decrease the appearance of deep vein thrombosis and decrease the mortality and morbidity in cancer patients. Secondly, vascular normalization of antiangiogenic agents can enhance the delivery and antitumor activity of chemotherapeutic by remodeling tumor vessels to a structural and functional phenotype more reflective of normal blood vessels and overcoming the physiological barriers to drug delivery within tumors through improvement in their functional efficiency. Thirdly, vascular promotion therapy can increase chemotherapy delivery and intracellular uptake of the drug by increasing tumor blood vessel density, blood flow, leakiness, and dilation (Figure 1).

Targeting tumor stroma to improve drug distribution in tumor

Any malignant tumor can erode the surrounding normal tissue, and the more erosive types of cancer have more destructive actions. If these cancer clusters erode adjacent normal or tumor vessels, microscopic hemorrhage may occur at any place and at any time within or adjacent to cancer tissues, and fibrin clots immediately form in situ to stop the bleeding. The fibrin clots are subsequently replaced by collagenous stroma in a process similar to that in normal wound healing and other non-malignant diseases. Fibrin clots formation in non-malignant disorders such as cardiac infarction, brain infarction, injuries and active rheumatoid arthritis should form only at the onset or active state of disease and subsequently disappear by plasmin digestion or replacement with collagen within a few weeks and is accompanied by some symptom. In the situation of so called ‘malignant cycle of blood coagulation’, the fibrin clot formation in cancer lasts for as long as the cancer cells survive in the body and occurs silently. It was hypothesized that cancer-induced blood coagulation may be an origin of tumor stroma and that fibrin clots in cancer tissues of patients who can receive chemotherapy are actually tumor-specific [40-42].

The kinetics of drug distribution within tumors are considered to be functions of interstitial conductivity, which is determined by the quantity and density of the extracellular matrix (e.g., proteoglycan, fibronectin), and fibrosis (e.g., col-
lager fibers) in the stroma [43-45]. Such compact assemblies of various tissue constituents in solid tumors cause reduced drug penetration and also act as a stromal barrier to prevent the diffusion of mAbs [43-48]. Yasuhiro Matsumura et al. have proposed the cancer stromal targeting (CAST) therapy using cytotoxic immunoconjugate [40, 42, 49]. There have been a few papers describing cancer stromal targeting therapy [50, 51].

**Reducing the interstitial fluid pressure to improve drug distribution in tumor**

The fact that the increased tumor IFP prevents the efficient uptake of therapeutic agents makes it important to find ways to increase the transvascular transport in tumors. Drug penetration into tumor tissue is inhibited by high interstitial fluid pressure (IFP); thus, reduction in tumor IFP might improve drug distribution [8]. All the compounds that interfere with the mechanisms responsible for high tumor IFP and solid stress could potentially promote the penetration of chemotherapeutics into the tumor bulk.

The reduction in tumor cell density caused by chemotherapy itself could decompress blood vessels, reduce microvascular pressure, and decrease IFP. Low-dose paclitaxel induces tumor cell apoptosis, which has been shown to reduce IFP and to enhance the delivery of paclitaxel to solid tumors [52, 53]. The concept that low-dose chemotherapy might cause limited cell killing but lead to reductions in tumor cell packing density and IFP sufficient to enhance the distribution of subsequent doses has been applied in the clinic. One randomized phase II study [54] demonstrated that paclitaxel reduced IFP and increased partial pressure of oxygen in breast cancer patients treated with neoadjuvant chemotherapy. The impact of this strategy on clinical outcome has not been evaluated and remains unclear.

The platelet-derived growth factor-beta receptor also mediates high tumor IFP, and imatinib, an antagonist of this receptor, might decrease IFP in tumors and thus enhance the therapeutic effects of chemotherapy [55, 56]. PDGF antagonists have also been shown to normalize the IFP of tumors. A selective low-molecular-weight inhibitor of the PDGF receptor kinases, imatinib was found to lower the IFP of the KAT-4 thyroid carcinoma grown in immunocompromised mice and of the PROb colon carcinoma grown in syngeneic rats [57]. The lowering of the tumor IFP was accompanied by an increased uptake of chemotherapeutic drugs, and an increased treatment effect [55, 56, 58]. As PDGF controls the IFP of normal connective tissue by promoting interactions between integrins of stromal fibroblasts with extracellular-matrix molecules and by stimulating contraction of these cells, it is likely that the ability of PDGF antagonists to block these effects will lower the IFP in tumors.

In animal models, it has been shown that this can be done by periodically increasing the systemic blood pressure, such as by infusion of angiotensin II, which leads to an increase in the hydrostatic pressure in capillaries and, therefore, a transiently increased gradient compared with the interstitium [59]. Using a soluble extracellular domain of the type II TGFβ receptor that efficiently binds and neutralizes two of the three TGFβ isoforms, Lammerts et al. [60] observed a decreased IFP of the KAT-4 thyroid carcinoma in mice. The effect was concentration dependent and was seen 5-10 days after administration of the TGFβ inhibitor. Injection of the hyaluronan-degrading enzyme hyaluronidase into human osteosarcoma xenografts reduced tumor IFP by 20-40% within 1 hour after injection; the IFP was restored to normal levels after 48 hours [61-63]. Tail-vein injection of the cytokine tumor necrosis factor-α (TNFα) in melanoma-bearing mice was found to cause a 50-70% decrease in tumor IFP [64]. Intraperitoneal administration of the anti-inflammatory corticosteroid dexamethasone into human osteosarcoma xenografts reduced tumor IFP by 20-40% within 1 hour after injection; the IFP was restored to normal levels after 48 hours [61-63]. Tail-vein injection of the cytokine tumor necrosis factor-α (TNFα) in melanoma-bearing mice was found to cause a 50-70% decrease in tumor IFP [64]. Intraperitoneal administration of the anti-inflammatory corticosteroid dexamethasone into immunodeficient mice that carry the human colon carcinoma tumor LSI74T was found to lower tumor IFP. The effect was seen after 4 days of treatment and was reversible, as it disappeared within 3 days after treatment was stopped [65]. Possible mechanisms for the effect of dexamethasone include a decrease in microvascular permeability and reduction of matrix molecule content. Bradykinin is a peptide that causes vasodilatation and increased vascular permeability. Stimulation of the G-protein-coupled bradykinin B2 receptor by the synthetic ligand Cereport (labradimil) was reported to increase the uptake of certain chemotherapeutics, such as [14C]carboplatin [21]. The increased uptake was rapid and was ascribed to a decreased IFP. Nicotinamide is the amide form of vitamin B3 and is known to
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sensitize tumors to radiotherapy, possibly through an increased oxygenation of tissues. Nicotinamide treatment was the first treatment found to lower tumor IFP; intraperitoneal injections of nicotinamide into mice bearing FSaII tumors lowered tumor IFP by about 40% within 1 hour [66]. The pro-inflammatory factor PGE1 causes swelling of normal tissues. Application of PGE1 to the subcutaneous tissue close to transplanted PROb tumors in immunocompetent BDIX rats led to a rapid decrease of tumor IFP by 30% [67]. PGE1 also increased the antitumor effect of 5-fluorouracil, but only when this drug was administered during the time IFP was lowered [68].

Targeting the tumor cells to improve drug distribution in tumor

The production of ATP-dependent drug transporters confers cellular resistance to different chemically unrelated chemotherapeutic agents. The use of reversing agents that inhibit the transporting function of these pumps would not only enable greater cellular uptake of drugs and increased sensitivity of perivascular tumor cells but also decrease the penetration of therapeutics into cells localized distant from vessels. The low penetration of drugs into the interstitium of deep tumor layers could be a cause of the limited efficacy of the multidrug resistance reversal agents in clinical trials. Inhibition of different types of pumps, such as the vacuolar H⁺-ATPases, could be a useful strategy for improving extracellular drug distribution. It has been verified that the use of proton pump inhibitors such as pantoprazole or omeprazole increases both extracellular and vesicle pH, thus decreasing the sequestration of basic chemotherapeutics. More drugs could enter the nucleus and cause cytotoxicity or exit the cell and be taken up by cells distant from blood vessels [69].

Another strategy that is used to favor drug distribution in tumor tissue is the reduction of the packing density of neoplastic cells. It has been demonstrated in animal tumor models that the administration of classic cytotoxic drugs can improve the penetration through the interstitial space of chemotherapeutics given in combination. Sequential cycles of chemotherapy lead to the sequential killing of cells at increasing distance from tumor blood vessels [45]. Taxanes are currently being investigated as potential distribution enhancers of other cytotoxicants. Several preclinical studies have observed that paclitaxel and docetaxel can reduce IFP and solid stress generated by hyperproliferating neoplastic cells, thus decompressing blood vessels and boosting the access of other therapeutic agents to the tumor tissue. Moschetta et al. [70] have shown that paclitaxel increases tumor perfusion, thus increasing the delivery of an antibody conjugated with interleukin-2 directed to xenografted melanomas. A clinical study partly confirmed these findings. In 25 breast cancer patients treated with neoadjuvant chemotherapy, paclitaxel (nine cycles of weekly paclitaxel at 80 mg/m²) succeeded in in increasing partial pressure of oxygen by almost 100% [71].

Changing molecular properties of a drug to improve drug distribution in tumor

One method to modify the pharmacokinetic properties of anticancer drugs is to incorporate them into macromolecular carriers such as liposomes or nanoparticles. In addition to the complex having a longer half-life than free drug in plasma, these large macromolecules are able to pass through fenestrations in the tumor blood vessels and release drug molecules into the interstitial space [72-74]. This strategy for transporting low-molecular-weight drugs can lead to higher efficacy than injection of the free drug [75]. One example of a conjugate drug is nab-paclitaxel, a nanoparticle formulation of paclitaxel bound to albumin, approved by the US Food and Drug Administration for therapy of breast cancer, non-small cell lung cancer, and pancreatic cancer. Differing from antibodies, albumin does not have a specific target, but its accumulation in tumor tissue is facilitated by the high binding affinity to secreted protein acid rich in cysteine, an extracellular glycoprotein that has been found to be overexpressed in many tumors. Desai et al. [76] demonstrated that the intratumor paclitaxel accumulation is 33% higher for nab-paclitaxel than for cremophor-based paclitaxel when each compound is administered at an equal dose. Moreover, the nab-paclitaxel formulation was better tolerated and had greater efficacy than the original drug.

Furthermore, based on the principle that a compound that specifically interacts with its target tends to be distributed where the target is preferentially expressed, the conjugation of a
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drug to an antibody can ameliorate its intratumor distribution. Coating the drug-carrying liposomes with antibodies to specific tumor antigens can facilitate the targeting of these macromolecular drug carriers to malignant cells [77]. The fact that folate receptor is highly expressed in ovarian cancer provided the rationale to develop a conjugate of folic acid with a very potent anticancer agent, desacetylvinblatine. The resulting conjugate drug, named vi-ntafolide, showed to be effective against ovarian carcinoma that expresses folate receptors because it is transported preferentially into receptor-carrying cancer cells; it is now under phase II/III clinical investigation [78].

Conclusion

Agents that improve drug delivery or activity by targeting the tumor microenvironment, especially in hypoxic regions of tumors, represent an important future direction for cancer therapy. The possibility of improving therapy of solid tumors by increasing the tumor uptake of chemotherapeutic agents or other therapeutics by improving the tumor microenvironment deserves further exploration.

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