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Review Article

Strategies to improve drug distribution in solid tumor

Huanrong Lan[1], Fangqin Lin[2], Qiuli Zhou[2], Liming Huang[1], Ketao Jin[2]

[1]Department of Breast and Thyroid Surgery, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, Shaoxing 312000, Zhejiang Province, P. R. China; [2]Department of Gastrointestinal Surgery, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, Shaoxing 312000, Zhejiang Province, P. R. China

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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

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.

Braykinin and angiotensin II are well-known physiological peptides exerting opposite vasodilatory 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-

genation 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-verpamil-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 angents 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-

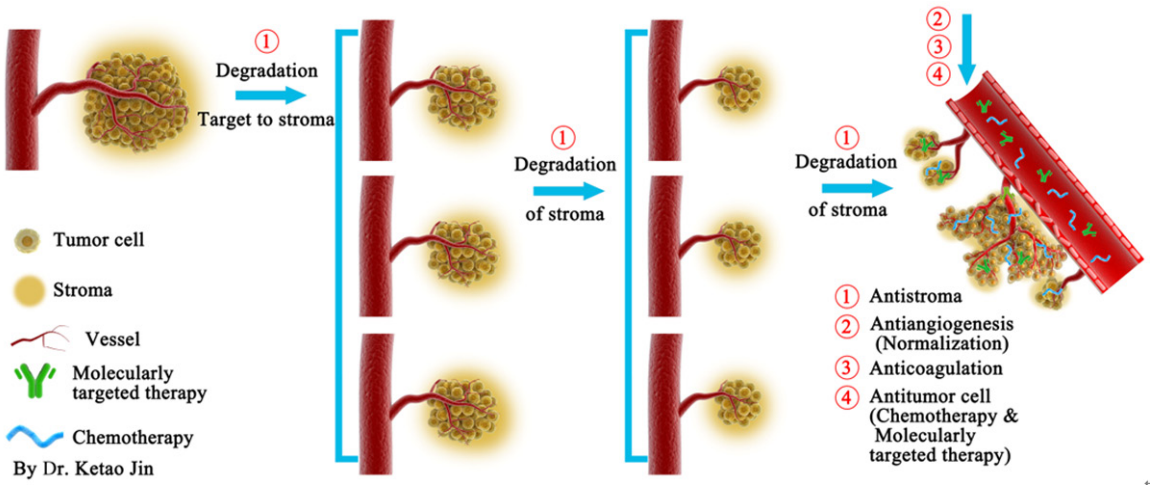

Figure 1. Strategies to improve drug distribution in solid tumor.

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 chemotherapy 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-

lagen 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 PDGFR 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 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

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/m^2) 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

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, desacetylvinblastine. The resulting conjugate drug, named vintafolide, 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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Address correspondence to: Dr. Liming Huang, Department of Breast and Thyroid Surgery, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, No. 568, Zhongxing North Road, Shaoxing 312000, Zhejiang Province, P. R. China. E-mail: lanhr2018@163.com; Dr. Ketao Jin, Department of Gastrointestinal Surgery, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, No. 568, Zhongxing North Road, Shaoxing 312000, Zhejiang Province, P. R. China. E-mail: jinketao2001@zju.edu.cn

References

[1] Moulder JE and Rockwell S. Tumor hypoxia: its impact on cancer therapy. Cancer Metastasis Rev 1987; 5: 313-341.

[2] Harris AL. Hypoxia--a key regulatory factor in tumour growth. Nat Rev Cancer 2002; 2: 38-47.

[3] Dean M, Fojo T and Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 2005; 5: 275-284.

[4] Leu AJ, Berk DA, Lymboussaki A, Alitalo K and Jain RK. Absence of functional lymphatics within a murine sarcoma: a molecular and functional evaluation. Cancer Res 2000; 60: 4324-4327.

[5] Jain RK, Munn LL and Fukumura D. Dissecting tumour pathophysiology using intravital microscopy. Nat Rev Cancer 2002; 2: 266-276.

[6] Jain RK. The Eugene M. Landis Award Lecture 1996. Delivery of molecular and cellular medicine to solid tumors. Microcirculation 1997; 4: 1-23.

[7] Milosevic MF, Fyles AW, Wong R, Pintilie M, Kavanagh MC, Levin W, Manchul LA, Keane TJ and Hill RP. Interstitial fluid pressure in cervical carcinoma: within tumor heterogeneity, and relation to oxygen tension. Cancer 1998; 82: 2418-2426.

[8] Heldin CH, Rubin K, Pietras K and Ostman A. High interstitial fluid pressure - an obstacle in cancer therapy. Nat Rev Cancer 2004; 4: 806-813.

[9] Folkman J. Angiogenesis: an organizing principle for drug discovery? Nat Rev Drug Discov 2007; 6: 273-286.

[10] Cesca M, Bizzaro F, Zucchetti M and Giavazzi R. Tumor delivery of chemotherapy combined with inhibitors of angiogenesis and vascular targeting agents. Front Oncol 2013; 3: 259.

[11] Jain RK. Normalizing tumor microenvironment to treat cancer: bench to bedside to biomarkers. J Clin Oncol 2013; 31: 2205-2218.

[12] Wildiers H, Guetens G, De Boeck G, Verbeken E, Landuyt B, Landuyt W, de Bruijn EA and van Oosterom AT. Effect of antivascular endothelial growth factor treatment on the intratumoral uptake of CPT-11. Br J Cancer 2003; 88: 1979-1986.

[13] Dickson PV, Hamner JB, Sims TL, Fraga CH, Ng CY, Rajasekeran S, Hagedorn NL, McCarville MB, Stewart CF and Davidoff AM. Bevacizumab-induced transient remodeling of the vasculature in neuroblastoma xenografts results in improved delivery and efficacy of systemically administered chemotherapy. Clin Cancer Res 2007; 13: 3942-3950.

[14] Carmeliet P and Jain RK. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. Nat Rev Drug Discov 2011; 10: 417-427.

[15] Batchelor TT, Sorensen AG, di Tomaso E, Zhang WT, Duda DG, Cohen KS, Kozak KR, Cahill DP, Chen PJ, Zhu M, Ancukiewicz M, Mrugala MM, Plotkin S, Drappatz J, Louis DN, Ivy P, Scadden

DT, Benner T, Loeffler JS, Wen PY and Jain RK. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell 2007; 11: 83-95.

[16] Huang Y, Yuan J, Righi E, Kamoun WS, Ancukiewicz M, Nezivar J, Santosuosso M, Martin JD, Martin MR, Vianello F, Leblanc P, Munn LL, Huang P, Duda DG, Fukumura D, Jain RK and Poznansky MC. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. Proc Natl Acad Sci U S A 2012; 109: 17561-17566.

[17] Webb T. Vascular normalization: study examines how antiangiogenesis therapies work. J Natl Cancer Inst 2005; 97: 336-337.

[18] Willett CG, Boucher Y, di Tomaso E, Duda DG, Munn LL, Tong RT, Chung DC, Sahani DV, Kalva SP, Kozin SV, Mino M, Cohen KS, Scadden DT, Hartford AC, Fischman AJ, Clark JW, Ryan DP, Zhu AX, Blaszkowsky LS, Chen HX, Shellito PC, Lauwers GY and Jain RK. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 2004; 10: 145-147.

[19] Feron O. Targeting the tumor vascular compartment to improve conventional cancer therapy. Trends Pharmacol Sci 2004; 25: 536-542.

[20] Emerich DF, Snodgrass P, Dean RL, Lafreniere D, Agostino M, Wiens T, Xiong H, Hasler B, Marsh J, Pink M, Kim BS and Bartus RT. Bradykinin modulation of tumor vasculature: I. Activation of B2 receptors increases delivery of chemotherapeutic agents into solid peripheral tumors, enhancing their efficacy. J Pharmacol Exp Ther 2001; 296: 623-631.

[21] Emerich DF, Dean RL, Snodgrass P, Lafreniere D, Agostino M, Wiens T, Xiong H, Hasler B, Marsh J, Pink M, Kim BS, Perdomo B and Bartus RT. Bradykinin modulation of tumor vasculature: II. activation of nitric oxide and phospholipase A2/prostaglandin signaling pathways synergistically modifies vascular physiology and morphology to enhance delivery of chemotherapeutic agents to tumors. J Pharmacol Exp Ther 2001; 296: 632-641.

[22] Thews O, Kelleher DK and Vaupel P. Disparate responses of tumour vessels to angiotensin II: tumour volume-dependent effects on perfusion and oxygenation. Br J Cancer 2000; 83: 225-231.

[23] Thews O, Hummel M, Kelleher DK, Lecher B and Vaupel P. Nifedipine improves blood flow and oxygen supply, but not steady-state oxygenation of tumours in perfusion pressure-controlled isolated limb perfusion. Br J Cancer 2002; 87: 1462-1469.

[24] Martinive P, De Wever J, Bouzin C, Baudelet C, Sonveaux P, Gregoire V, Gallez B and Feron O. Reversal of temporal and spatial heterogeneities in tumor perfusion identifies the tumor vascular tone as a tunable variable to improve drug delivery. Mol Cancer Ther 2006; 5: 1620-1627.

[25] Sonveaux P, Dessy C, Martinive P, Havaux X, Jordan BF, Gallez B, Gregoire V, Balligand JL and Feron O. Endothelin-1 is a critical mediator of myogenic tone in tumor arterioles: implications for cancer treatment. Cancer Res 2004; 64: 3209-3214.

[26] Kaanders JH, Bussink J and van der Kogel AJ. ARCON: a novel biology-based approach in radiotherapy. Lancet Oncol 2002; 3: 728-737.

[27] Gupta N, Saleem A, Kotz B, Osman S, Aboagye EO, Phillips R, Vernon C, Wasan H, Jones T, Hoskin PJ and Price PM. Carbogen and nicotinamide increase blood flow and 5-fluorouracil delivery but not 5-fluorouracil retention in colorectal cancer metastases in patients. Clin Cancer Res 2006; 12: 3115-3123.

[28] Brunstein F, Rens J, van Tiel ST, Eggermont AM and ten Hagen TL. Histamine, a vasoactive agent with vascular disrupting potential, improves tumour response by enhancing local drug delivery. Br J Cancer 2006; 95: 1663-1669.

[29] Martinive P, Defresne F, Bouzin C, Saliez J, Lair F, Gregoire V, Michiels C, Dessy C and Feron O. Preconditioning of the tumor vasculature and tumor cells by intermittent hypoxia: implications for anticancer therapies. Cancer Res 2006; 66: 11736-11744.

[30] Ansiaux R, Baudelet C, Cron GO, Segers J, Dessy C, Martinive P, De Wever J, Verrax J, Wauthier V, Beghein N, Gregoire V, Buc Calderon P, Feron O and Gallez B. Botulinum toxin potentiates cancer radiotherapy and chemotherapy. Clin Cancer Res 2006; 12: 1276-1283.

[31] Wong PP, Demircioglu F, Ghazaly E, Alrawashdeh W, Stratford MR, Scudamore CL, Cereser B, Crnogorac-Jurcevic T, McDonald S, Elia G, Hagemann T, Kocher HM and Hodivala-Dilke KM. Dual-action combination therapy enhances angiogenesis while reducing tumor growth and spread. Cancer Cell 2015; 27: 123-137.

[32] De Cicco M. The prothrombotic state in cancer: pathogenic mechanisms. Crit Rev Oncol Hematol 2004; 50: 187-196.

[33] Varki A. Trousseau's syndrome: multiple definitions and multiple mechanisms. Blood 2007; 110: 1723-1729.

[34] Caine GJ, Stonelake PS, Lip GY and Kehoe ST. The hypercoagulable state of malignancy: pathogenesis and current debate. Neoplasia 2002; 4: 465-473.

[35] Levine M and Hirsh J. The diagnosis and treatment of thrombosis in the cancer patient. Semin Oncol 1990; 17: 160-171.
[36] Mousa SA. Low-molecular-weight heparin in thrombosis and cancer. Semin Thromb Hemost 2004; 30 Suppl 1: 25-30.
[37] Rickles FR and Falanga A. Molecular basis for the relationship between thrombosis and cancer. Thromb Res 2001; 102: V215-224.
[38] Chia WK, Ali R and Toh HC. Aspirin as adjuvant therapy for colorectal cancer--reinterpreting paradigms. Nat Rev Clin Oncol 2012; 9: 561-570.
[39] Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, Imamura Y, Qian ZR, Baba Y, Shima K, Sun R, Nosho K, Meyerhardt JA, Giovannucci E, Fuchs CS, Chan AT and Ogino S. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med 2012; 367: 1596-1606.
[40] Yasunaga M, Manabe S, Tarin D and Matsumura Y. Cancer-stroma targeting therapy by cytotoxic immunoconjugate bound to the collagen 4 network in the tumor tissue. Bioconjug Chem 2011; 22: 1776-1783.
[41] Yasunaga M, Manabe S and Matsumura Y. New concept of cytotoxic immunoconjugate therapy targeting cancer-induced fibrin clots. Cancer Sci 2011; 102: 1396-1402.
[42] Matsumura Y. Cancer stromal targeting (CAST) therapy. Adv Drug Deliv Rev 2012; 64: 710-719.
[43] Tredan O, Galmarini CM, Patel K and Tannock IF. Drug resistance and the solid tumor microenvironment. J Natl Cancer Inst 2007; 99: 1441-1454.
[44] Ghajar CM and Bissell MJ. Extracellular matrix control of mammary gland morphogenesis and tumorigenesis: insights from imaging. Histochem Cell Biol 2008; 130: 1105-1118.
[45] Minchinton AI and Tannock IF. Drug penetration in solid tumours. Nat Rev Cancer 2006; 6: 583-592.
[46] Ricart AD and Tolcher AW. Technology insight: cytotoxic drug immunoconjugates for cancer therapy. Nat Clin Pract Oncol 2007; 4: 245-255.
[47] Saito Y, Yasunaga M, Kuroda J, Koga Y and Matsumura Y. Enhanced distribution of NK012, a polymeric micelle-encapsulated SN-38, and sustained release of SN-38 within tumors can beat a hypovascular tumor. Cancer Sci 2008; 99: 1258-1264.
[48] Mahadevan D and Von Hoff DD. Tumor-stroma interactions in pancreatic ductal adenocarcinoma. Mol Cancer Ther 2007; 6: 1186-1197.
[49] Matsumura YYasunaga M, Manabe S and Matsumura Y. New concept of cytotoxic immunoconjugate therapy targeting cancer-induced fibrin clots. Cancer Sci 2011; 102: 1396-1402.
[50] Ostermann E, Garin-Chesa P, Heider KH, Kalat M, Lamche H, Puri C, Kerjaschki D, Rettig WJ and Adolf GR. Effective immunoconjugate therapy in cancer models targeting a serine protease of tumor fibroblasts. Clin Cancer Res 2008; 14: 4584-4592.
[51] Palumbo A, Hauler F, Dziunycz P, Schwager K, Soltermann A, Pretto F, Alonso C, Hofbauer GF, Boyle RW and Neri D. A chemically modified antibody mediates complete eradication of tumours by selective disruption of tumour blood vessels. Br J Cancer 2011; 104: 1106-1115.
[52] Griffon-Etienne G, Boucher Y, Brekken C, Suit HD and Jain RK. Taxane-induced apoptosis decompresses blood vessels and lowers interstitial fluid pressure in solid tumors: clinical implications. Cancer Res 1999; 59: 3776-3782.
[53] Jang SH, Wientjes MG and Au JL. Enhancement of paclitaxel delivery to solid tumors by apoptosis-inducing pretreatment: effect of treatment schedule. J Pharmacol Exp Ther 2001; 296: 1035-1042.
[54] Taghian AG, Abi-Raad R, Assaad SI, Casty A, Ancukiewicz M, Yeh E, Molokhia P, Attia K, Sullivan T, Kuter I, Boucher Y and Powell SN. Paclitaxel decreases the interstitial fluid pressure and improves oxygenation in breast cancers in patients treated with neoadjuvant chemotherapy: clinical implications. J Clin Oncol 2005; 23: 1951-1961.
[55] Pietras K, Rubin K, Sjoblom T, Buchdunger E, Sjoquist M, Heldin CH and Ostman A. Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. Cancer Res 2002; 62: 5476-5484.
[56] Pietras K, Stumm M, Hubert M, Buchdunger E, Rubin K, Heldin CH, McSheehy P, Wartmann M and Ostman A. STI571 enhances the therapeutic index of epothilone B by a tumor-selective increase of drug uptake. Clin Cancer Res 2003; 9: 3779-3787.
[57] Pietras K, Ostman A, Sjoquist M, Buchdunger E, Reed RK, Heldin CH and Rubin K. Inhibition of platelet-derived growth factor receptors reduces interstitial hypertension and increases transcapillary transport in tumors. Cancer Res 2001; 61: 2929-2934.
[58] Pietras K. Increasing tumor uptake of anticancer drugs with imatinib. Semin Oncol 2004; 31: 18-23.
[59] Netti PA, Hamberg LM, Babich JW, Kierstead D, Graham W, Hunter GJ, Wolf GL, Fischman A, Boucher Y and Jain RK. Enhancement of fluid filtration across tumor vessels: implication for delivery of macromolecules. Proc Natl Acad Sci U S A 1999; 96: 3137-3142.
[60] Lammerts E, Roswall P, Sundberg C, Gotwals PJ, Koteliansky VE, Reed RK, Heldin NE and Rubin K. Interference with TGF-beta1 and -beta3 in tumor stroma lowers tumor intersti-

tial fluid pressure independently of growth in experimental carcinoma. Int J Cancer 2002; 102: 453-462.
[61] Brekken C, Hjelstuen MH, Bruland OS and de Lange Davies C. Hyaluronidase-induced periodic modulation of the interstitial fluid pressure increases selective antibody uptake in human osteosarcoma xenografts. Anticancer Res 2000; 20: 3513-3519.
[62] Brekken C, Bruland OS and de Lange Davies C. Interstitial fluid pressure in human osteosarcoma xenografts: significance of implantation site and the response to intratumoral injection of hyaluronidase. Anticancer Res 2000; 20: 3503-3512.
[63] Brekken C and de Lange Davies C. Hyaluronidase reduces the interstitial fluid pressure in solid tumours in a non-linear concentration-dependent manner. Cancer Lett 1998; 131: 65-70.
[64] Kristensen CA, Nozue M, Boucher Y and Jain RK. Reduction of interstitial fluid pressure after TNF-alpha treatment of three human melanoma xenografts. Br J Cancer 1996; 74: 533-536.
[65] Kristjansen PE, Boucher Y and Jain RK. Dexamethasone reduces the interstitial fluid pressure in a human colon adenocarcinoma xenograft. Cancer Res 1993; 53: 4764-4766.
[66] Lee I, Boucher Y and Jain RK. Nicotinamide can lower tumor interstitial fluid pressure: mechanistic and therapeutic implications. Cancer Res 1992; 52: 3237-3240.
[67] Rubin K, Sjoquist M, Gustafsson AM, Isaksson B, Salvessen G and Reed RK. Lowering of tumoral interstitial fluid pressure by prostaglandin E(1) is paralleled by an increased uptake of (51)Cr-EDTA. Int J Cancer 2000; 86: 636-643.
[68] Salnikov AV, Iversen VV, Koisti M, Sundberg C, Johansson L, Stuhr LB, Sjoquist M, Ahlstrom H, Reed RK and Rubin K. Lowering of tumor interstitial fluid pressure specifically augments efficacy of chemotherapy. FASEB J 2003; 17: 1756-1758.
[69] Tunggal JK, Cowan DS, Shaikh H and Tannock IF. Penetration of anticancer drugs through solid tissue: a factor that limits the effectiveness of chemotherapy for solid tumors. Clin Cancer Res 1999; 5: 1583-1586.
[70] Kyle AH, Huxham LA, Chiam AS, Sim DH and Minchinton AI. Direct assessment of drug penetration into tissue using a novel application of three-dimensional cell culture. Cancer Res 2004; 64: 6304-6309.
[71] Hicks KO, Pruijn FB, Baguley BC and Wilson WR. Extravascular transport of the DNA intercalator and topoisomerase poison N-[2-(Dimethylamino)ethyl]acridine-4-carboxamide (DACA): diffusion and metabolism in multicellular layers of tumor cells. J Pharmacol Exp Ther 2001; 297: 1088-1098.
[72] Di Paolo A and Bocci G. Drug distribution in tumors: mechanisms, role in drug resistance, and methods for modification. Curr Oncol Rep 2007; 9: 109-114.
[73] Dreher MR, Liu W, Michelich CR, Dewhirst MW, Yuan F and Chilkoti A. Tumor vascular permeability, accumulation, and penetration of macromolecular drug carriers. J Natl Cancer Inst 2006; 98: 335-344.
[74] Di Paolo A. Liposomal anticancer therapy: pharmacokinetic and clinical aspects. J Chemother 2004; 16 Suppl 4: 90-93.
[75] Tang N, Du G, Wang N, Liu C, Hang H and Liang W. Improving penetration in tumors with nanoassemblies of phospholipids and doxorubicin. J Natl Cancer Inst 2007; 99: 1004-1015.
[76] Hicks KO, Pruijn FB, Sturman JR, Denny WA and Wilson WR. Multicellular resistance to tirapazamine is due to restricted extravascular transport: a pharmacokinetic/pharmacodynamic study in HT29 multicellular layer cultures. Cancer Res 2003; 63: 5970-5977.
[77] Kontermann RE. Immunoliposomes for cancer therapy. Curr Opin Mol Ther 2006; 8: 39-45.
[78] Kyle AH and Minchinton AI. Measurement of delivery and metabolism of tirapazamine to tumour tissue using the multilayered cell culture model. Cancer Chemother Pharmacol 1999; 43: 213-220.