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Review Article

Anti-angiogenesis or pro-angiogenesis for cancer treatment: focus on drug distribution

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Abstract: Enhancing chemotherapy delivery to tumors, improving tumor growth control, reducing metastasis, and increasing survival are all critical objectives of improved cancer therapy. One of the obstacles to the success of anticancer therapies is related to the inefficient distribution of drugs to tumor cells. To be effective, chemotherapeutics must reach a concentration in cancer cells that is sufficient to inhibit its targets. In the past years, the vascular normalization theory has gained widespread acceptance for explaining additional antitumor effects of inhibitors of vascular endothelial growth factor (VEGF) signaling, when combined with chemotherapeutics. Vascular normalization is a strategy to enhance the antitumor effects of chemotherapeutics, but this is time and dose dependent and therefore difficult to implement clinically. Thus, alternative strategies that overcome these issues are needed. Accumulating scientific data demonstrate an alternative approach called "vascular promotion therapy" can increase chemotherapeutics delivery and intracellular uptake of the drug and reduces hypoxia by increasing tumor blood vessel density, blood flow, leakiness, and dilation, which leads to reduced cancer growth and metastasis. In this article, we first summarize the structural and functional abnormalities of the tumor microvasculature to highlight the importance of this phenomenon for chemotherapeutics distribution. Next, we summarize the limitations of anti-angiogenic strategy in cancer treatment, discuss some key prototypical underlying mechanisms of vascular normalization and initial clinical evidence of vascular promotion therapy, and speculate on the clinical potential of anticoagulation as a novel paradigm to improve cancer treatment.

Keywords: Antiangiogenesis, -proangiogenesis, anticoagulation, drug distribution

Introduction

Enhancing chemotherapy delivery to tumors, improving tumor growth control, reducing metastasis, and increasing survival are all critical objectives of improved cancer therapy. One of the obstacles to the success of anticancer therapies is related to the inefficient distribution of drugs to cancer cells. To be effective, chemotherapeutics must reach a concentration in cancer cells that is sufficient to inhibit its targets. 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 one of the key reasons for low delivery can be mainly ascribed to the tumor abnormal blood vessels, which constitutes a key obstacle to the homogeneous distribution of chemotherapeutics to the tumor tissue.

In the majority of cancers, vessel growth is not only stimulated, but these vessels are also abnormal in almost all aspects of their structure and function [1-4]. This results in a hostile tumor microenvironment-characterized by hypoxia, low pH and high interstitial hostile fluid pressure-that can alter the intrinsic characteristics of tumor cells such that malignant tumor clones are selected and escape of tumor cells through leaky vessels is facilitated [2]. Abnormal tumor vessels can also impede the function of immune cells in tumors, as well as the transport and/or distribution of chemotherapeutics and oxygen. As a result, the abnormal tumor vascu-

lature can also lead to a resistance of tumor cells to radiation therapy and many chemotherapeutics. In addition, hypoxia upregulates the production of angiogenic factors by cancer and stromal cells, which further aggravate vessel disorganization and thereby fuel non-productive angiogenesis in an endless self-reinforcing loop.

For more than a decade, one of the approaches in cancer treatment has been targeting angiogenesis-the development of new blood vessels from pre-existing ones in the tumor microenvironment. Significant efforts have focused on anti-angiogenic strategies aimed at reducing tumor blood vessel density to inhibit tumor growth [5]. Unfortunately, anti-angiogenic strategies have not been very successful in the clinic and multiple factors have been found to contribute to increased tumor aggressiveness and therapy resistance following this kind of treatment. Anti-angiogenic agents can increase tumor hypoxia, promoting the selection of more-aggressive cancer cells that can proliferate under oxygen-deficient conditions, and reduce chemotherapy delivery, for instance.

However, increasing evidence [6, 7] suggests that vascular endothelial growth factor (VEGF) inhibitors can restore a balance between pro- and anti-angiogenic cytokines, tumor vessels, at least transiently, display a structural and functional phenotype more reflective of normal blood vessels [8]. This process, termed vascular normalization, remodels tumor vessels and partially overcomes the physiological barriers to drug and oxygen delivery within tumors through improvement in their functional efficiency, thus enhancing the delivery and antitumor activity of chemotherapy and radiation. However, this process of vascular normalization seems to be transient with a relatively narrow window during which synergy is likely to be achieved, and after which, the tumor vasculature is destroyed. Vascular normalization is a strategy to enhance the antitumor effects of chemotherapeutics, but this is time and dose dependent and therefore difficult to implement clinically. Thus, alternative strategies that overcome these issues are needed. Accumulating scientific dada demonstrate an alternative approach called “vascular promotion therapy” can increase chemotherapy delivery and intracellular uptake of the drug and reduces hypoxia by increasing tumor blood vessel density, blood flow, leakiness, and dilation, which leads to reduced cancer growth and metastasis.

In this article, we first summarize the structural and functional abnormalities of the tumor microvasculature to highlight the importance of this phenomenon for chemotherapeutics distribution. Next, we summarize the limitations of anti-angiogenesis in cancer treatment, discuss some key prototypical underlying mechanisms of vascular normalization and initial clinical evidence of vascular promotion therapy, and speculate on the clinical potential of anticoagulation as a novel paradigm to improve cancer treatment.

Abnormality of tumor vasculature

Classic laws that explain hemodynamic and transcapillary flow can only in part explain the permeation of drugs into tumor tissue. Fick’s law, which states that the diffusion rate of small molecules depends on their concentration gradient, and Starling’s forces, which describe the capillary filtration movement of fluids and macromolecules [9, 10] are useful mathematical means to define the extravasation of drugs and their distribution in tissue but are not exhaustive for a situation as complex as that of tumors.

Tumor vessels are tortuous; they follow a serpentine course, branch irregularly in a chaotic network of tangles, connect to one another randomly and criss-cross the stroma haphazardly [2-4, 11]. They are also strikingly heterogeneous and exhibit a spectrum of vesselsubtypes, ranging from capillaries and ‘mother’ vessels (big, leaky, thin-walled, pericyte-depleted fenestrated sinusoids) to glomeruloid vessel outgrowths and vascular malformations [4, 12]. Vessel diameters are uneven, because their wall is compressed by tumor or stromal cells, with some vessels being oversized, others being more immature smaller vessels, while in other areas, vessels are lacking altogether [13, 14].

Structural anomalies of the vessel wall, such as large gaps between endothelial cells, defects in pericyte coverage and function, discontinuous or absent basement membranes, and the presence of pro-angiogenic molecules such as VEGF, contribute to the increased permeability of the tumor vessels [15]. The direct consequences are a slowing down of blood flow veloc-

ity and an increased outflow of plasma macromolecules (e.g., fibrinogen), leading to a high oncotic pressure in the interstitium [10]. The complexity of this scenario is increased by the fact that vascular permeability varies spatially and temporally within the same tumor and between different tumors [15]. Furthermore, the capillary network is structurally abnormal and tortuous, with several loops and arteriovenous shunts, and some vessels are compressed by the growing tumor cells. This causes an irregular blood flow, changed hematocrit, and hemorrhages. Another aspect of heterogeneity in intratumoral blood flow is the so-called intermittent hypoxia [9]. It consists of reversible functional alterations in tumor blood vessels, and it seems to be caused by rapid fluctuations in hematocrit, temporary stagnation, local vascular remodeling, and alterations in vascular tone [9].

As a consequence, the delivery of not only blood-borne drugs but also oxygen and nutrients, as well as the clearance of products of metabolism, are reduced in some regions of the tumor tissue, which become hypoxic and acidic [16]. It is widely recognized that hypoxia and poor nutrition induce a more malignant phenotype of cancer cells through genetic and epigenetic mechanisms [17]. Therefore, the antineoplastic agents are not only physically limited in their access to the tumor bulk but are also compromised in their activity because cells in hypoxic, acidic, and nutrient-deprived regions engage resistance mechanisms [17].

Limitations of anti-angiogenesis therapy in cancer

The seminal work by Folkman on tumor angiogenesis stimulated the discovery and development of many angiogenesis inhibitors [18]. The most validated anti-angiogenic strategies act on the VEGF axis, blocking VEGF directly with the neutralizing antibody bevacizumab or the aflibercept (VEGF trap), or indirectly with low-molecular-weight tyrosine kinase VEGF receptor inhibitors (e.g., sunitinib, sorafenib, and pazopanib) [19].

Although promising results have been achieved preclinically and clinically, improvements to current anti-angiogenic therapies are still ongoing [20]. Some anti-angiogenic drugs have shown antitumor activity, mostly in combination with chemotherapeutics, but the mechanism of the increased efficacy when given in combination has not been fully elucidated. For example, some data suggest that anti-angiogenic therapy can increase intratumoral hypoxia, leading to radioresistance and chemoresistance as well as potentially increasing metastasis, at least in mouse models [21, 22]. In terms of drug delivery, one would expect that the anti-angiogenic treatment, by altering tumor vasculature, impairs the delivery of chemotherapy. In fact, reducing angiogenesis did impair drug delivery to the tumor, ultimately restricting its efficacy [23]. The fact that the anti-angiogenic drugs enhance the response to anticancer drugs when given in combination suggests that they do not necessarily decrease drug delivery to tumor tissue. Jain [15] has proposed that anti-angiogenic 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 interstitial fluid pressure (IFP) and reduction of hypoxia, which provides an improvement of the penetration and activity of concurrent cytotoxic agents. Robust experimental evidence supporting this theory is still lacking, and there are conflicting data in different preclinical models.

In support of the normalization hypothesis, Wildiers et al. [24] 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 intratumoral irinotecan concentration. Dickson et al. [25] 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 intratumoral 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 [25]. These findings are consistent with the hypothesis that the effect of the anti-angiogenic therapy is transient, generating a narrow window of time during which synergy can be achieved. These effects seem to be limited not only temporally but also spatially: acting on a heterogeneous tissue, normalization probably occurs only in some regions of the tumor, where anti-angiogenic agents succeed in correcting the imbalance

between pro- and anti-angiogenic factors. Consequently, it is of the utmost importance to carefully define the timing of the normalization window, the scheduling, and the dosing of anti-angiogenic therapies in order to optimize the efficacy of a combination of antitumor strategies. Pastuskovas et al. [26] reported that in human epidermal growth factor receptor-2 (HER-2) expressing breast cancer xenografts, bevacizumab causes reduced tumor uptake of trastuzumab, a monoclonal antibody directed against HER-2, probably because of the blood flow and vascular permeability reduction. Cesca et al. [27] and Bello et al. [28] investigated the tumor concentrations of paclitaxel given in combination with small-molecular tyrosine kinase inhibitors with an anti-angiogenic effect in xenograft models. It was found that the tumor concentration of paclitaxel decreases when the anti-angiogenic compound is administered before paclitaxel. Nevertheless, the combined treatment improved antitumor activity. Chauhan et al. [29] reported that in murine models of breast cancer, blocking VEGF receptor 2 with an antibody increases the delivery of nanoparticles of 12 nm diameter while it hampers the delivery of larger nanoparticles (125 nm diameter). They suggested that the anti-VEGF treatment causes a reduction in size of the holes in tumor vasculature, thus producing discrepant effects on extravasation of molecules depending on their size. The available clinical information on the influence of anti-angiogenic drugs on the distribution of anticancer drugs given in combination is limited. Willet et al. [7] 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. Unfortunately, no data on the tumor concentration of 5-fluorouracil were reported. In another clinical setting, Van der Veldt et al. [23] obtained discrepant results. In 10 non-small cell lung cancer patients, the administration of bevacizumab (15 mg/kg, infused over 90 min) reduced both perfusion and net influx rate of $^{[11C]}$ docetaxel as visualized by positron emission tomography (PET). The effect lasted from 5 h to 4 days after infusion of bevacizumab. Supporters of the normalization theory claim that this treatment schedule constitutes excessive or unduly prolonged dosing of bevacizumab, which can lead to rapid vascular regression or to the activation of alternative pro-angiogenic pathways, thus hampering docetaxel delivery. This counter-productive effect could also be responsible for the lack of efficacy of bevacizumab given in combination in phase III breast cancer trials.

Pro-angiogenesis in cancer treatment

Vascular normalization, using anti-angiogenic agents, is the process by which partial loss of blood vessel density is associated with a temporary increase in blood flow [30]. This approach has shown significant promise [6, 31], 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 [32]. Thus, the paradigms that underlie anti-angiogenic and vascular normalization strategies are still open for improvement. Designing vascular modulation strategies that overcome at least some of these issues is highly desirable.

With these issues in mind, Wong et al. [33] have proposed an alternative approach called “vascular promotion therapy”. Low doses of the anti-angiogenic 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. To that end, they assessed the ability of these agents to inhibit tumor growth and metastasis in a xenograft mouse model of lung cancer and in a mouse model of spontaneous pancreatic cancer. Whereas treatment with gemcitabine alone had no apparent effect compared with placebo, treatment with the triple combination of cilengitide-verpamil-gemcitabine reduced tumor burden and number of metastases considerably, and these effects were sustained after cessation of treatment. Analysis of the tumor vasculature showed that the triple combination 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, therefore suggesting a potential enhanced intratumoral drug delivery. Indeed, gemcitabine concentrations in the whole tumor

in vivo were higher when administered in combination with cilengitide and verapamil. The authors then investigated whether this increase in blood vessel density, dilation, perfusion and permeability resulted in increased delivery of gemcitabine. Gemcitabine uptake into cells is regulated by equilibrative nucleoside transporter 1 (ENT1) and ENT2, as well as by concentrative nucleoside transporter 3 (CNT3), which mediates the unidirectional flow of the drug into the cell. Once inside the cell, gemcitabine is metabolized by deoxycytidine kinase (DCK). In vitro experiments showed that treatment with the triple combination increased the expression levels of ENT1 and ENT2, as well as the expression of CNT3, which increased the influx of gemcitabine. Low doses of cilengitide also increased DCK expression, thus increasing 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 in cancer treatment: perspectives

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 [34, 35]. 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 [35-37]. Anticancer therapy (i.e., surgery/chemotherapy/hormone therapy) may significantly increase the risk of thromboembolic events by the mechanisms, such as procoagulant release, endothelial damage, or stimulation of tissue factor production by host cells [35]. Chemotherapy can increase the risk of thrombosis in cancer patients. This has been best studied in breast cancer, where tamoxifen and cytotoxic chemotherapy both appear independently to increase the risk of venous thrombosis [35]. Acute arterial thrombotic events were also reported to be induced by cytotoxic chemotherapy especially in patients receiving platinum-based chemotherapy [38, 39]. The impact of cancer cells and chemotherapy on the activation of the coagulation cascade is responsible for a prothrombotic state found in many cancer patients [40]. Various mechanisms related to the activation of the coagulation or fibrinolytic systems in cancer may be involved in tumor development, progression and metastasis. Activation of coagulation can have both systemic and local consequences. The systemic consequences involve deep vein thrombosis or metastasis. Local consequences involve the deposition of fibrin and plasma proteins in the tumor interstitium, resulting at least in part, from tumor vasculature that is inherently leaky. This fibrin deposition results in imposition of the initial tumor structure, regulation of inflammatory cell infiltration, induction of angiogenesis and formation of a mature stroma. In addition, accumulation of fibrin and other plasma proteins in the tumor microenvironment contributes significantly to increased interstitial pressure that impedes the penetration of chemotherapeutic agents into the tumor [8, 41, 42]. Tumor generated polymerized fibrin also results in the formation of a physical barrier protecting the tumor from natural killer cells and other exogenous anticancer agents.

Although vascular normalization of anti-angiogenic 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 therapy 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 [43, 44].

Herein, we hypothesize that anticoagulation therapy in combination with vascular normalization of anti-angiogenic 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 anti-angiogenic 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.

Conclusion

Vascular normalization of VEGF inhibitors is a strategy to enhance the antitumor effects of chemotherapeutics, but this is time and dose dependent and therefore difficult to implement clinically. Vascular promotion therapy and anticoagulation therapy are now emerging as novel opportunities to improve the efficacy of anticancer therapy. A greater awareness of the concept of vascular promotion and anticoagulation in human cancer will further stimulate interest in studying these processes. Though the therapeutic benefits of vascular promotion and anticoagulation in human cancer remain only indirectly proven to date, emerging preclinical evidence would increasingly support the paradigm that vascular promotion and anticoagulation strategies can be beneficial. None-theless, many challenges remain to be add-ressed in order to fully exploit the therapeutic potential of vascular promotion and anticoagulation in cancer patients.

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Disclosure of conflict of interest

None.

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