Anti-Angiogenic Therapeutic Drugs for Treatment of Human Cancer

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One of the proposed benefits of targeted therapies is reduced toxicity and improved quality of life. The approval of a new broad family of molecularly targeted anticancer drugs represents one of the most significant recent advances in clinical oncology. The growth of solid tumors is dependent on their capacity to acquire a blood supply. Much effort has been directed towards the development of drugs that disrupt or normalize this process for cancer therapy. Some tumors also contain vasculogenic mimicry channels consisting of cancer cells and their extracellular matrix. In solid tumors, vasculogenic mimicry has been found to be strongly correlated with advanced-stage disease and poor outcome. In this review article, we summarize the clinical use of small molecules and therapeutic antibodies as angiogenesis inhibitors and their toxic side effects. We discuss vasculogenic mimicry of solid tumors and describe strategies for identifying putative tumor vascular targets. We end by discussing the future prospects for the clinical use of vascular targeting in treatment of cancer.

Keywords:
- angiogenesis
- anti-angiogenic therapy
- vasculogenic mimicry
- toxicity


Introduction

Chemotherapeutic agents do not specifically target tumor cells, but rather interfere with cell division or inhibit enzymes involved DNA replication or metabolism. These drugs therefore also damage the normal dividing cells of rapidly regenerating tissues, such as those of the bone marrow, gut mucosa and hair follicles. Cancer chemotherapy is limited by a lack of specificity, resulting in damage to not only cancer cells but also normal cells. This creates a narrow therapeutic index. Trying to avoid the side effects occurring as a result of toxicities to normal tissues, we often give suboptimal doses of cancer chemotherapeutic agents. The result is often incomplete tumor response and eventual failure of therapy, early disease relapse, drug resistance, and metastatic disease. The growth of solid tumors is dependent on their capacity to induce angiogenesis, to induce the growth of blood vessels to supply them with oxygen and nutrients. Considering the side effects associated with traditional chemotherapies and the possibility of interrupting a tumor’s supply of oxygen and nutrient, there has been great interest taken in the targeting of tumor vasculature and much effort has been directed towards the development of anti-angiogenic agents that could disrupt this angiogenesis. Administration of the maximum tolerated dose (MTD) is usually associated with maximum clinical benefit. The anti-

angiogenic efficacy of chemotherapy seems to be optimized by administering comparatively low doses of drug on a frequent or continuous schedule, referred to as metronomic chemotherapy. In addition to reduced acute toxicity, the efficacy of metronomic chemotherapy seems to increase when administered in combination with specific antiangiogenic drugs. Toxicities may be caused or depend on the duration of the treatment and different rates of antiangiogenic onset. Bevacizumab has been associated with gastrointestinal perforations and wound-healing complications and found to possibly cause acute life-threatening problems [1]. Meanwhile, anthraciclyne chemotherapy may reduce the left ventricular ejection fraction (LVEF), which can also ultimately be life threatening [2,3].

Although it was originally thought that tumor cells would not likely develop the resistance to anti-angiogenic therapy, in almost all treated patients tumors eventually become resistant to angiogenesis inhibitors [4,5]. To avoid the effect of these inhibitors, tumors may activate alternative pathways by increasing redundancy of angiogenic factors, activating other signaling pathways, and vasculogenic mimicry. The inhibitors might induce a reciprocal increase in expression of the growth factor or its receptor (i.e., VEGF or VEGFR) and provoke toxicity of these agents.

One possible approach to improve the therapeutic efficacy and selective toxicity of anticancer drugs is by targeting anticancer drugs through monoclonal antibodies (MAbs) or peptide ligands that bind to molecules that are overexpressed on the plasma membrane of cancer cells or tumor-associated endothelial cells. Ligand-targeted therapy will make possible better specificity and limit toxicity and shows promise in the development of new therapies for cancer.

Anti-angiogenic therapeutic drugs

Tumor blood vessels are distinct from normal resting blood vessels, and the distinctness of these special tumor
vessels features them as good targets for cancer therapies. In order to block tumor growth and metastasis formation, a number of inhibitors targeting the tumor vasculature have been identified in in vitro and in vivo anti-angiogenesis studies [6]. Anti-angiogenic therapeutic drugs may act by inhibiting synthesis of angiogenic proteins by cancer cells, neutralizing the angiogenic proteins, inhibiting the receptors of endothelia for angiogenic proteins, or directly inducing endothelial cell apoptosis (Figure 1). These inhibitors include therapeutic antibodies and small molecules both capable of targeting angiogenic growth factors, such as VEGF and bFGF, or angiogenic growth factor receptors, such as VEGFR and PDGFR.

The anti-angiogenic efficacy of chemotherapy is better observed when administering comparatively low doses of a chemotherapeutic agent on a frequent or continuous schedule. This approach, called metronomic chemotherapy refers to the frequent administration of chemotherapeutic agents at doses significantly below the MTD with no prolonged drug-free breaks [7,8]. Browder et al. [7] first reported that the main targets of metronomic chemotherapy are the endothelial cells involved in the expanding vasculature of a tumor. In addition to reduced acute toxicity, the efficacy of metronomic chemotherapy seems to increase when administered in combination with specific anti-angiogenic therapeutic agents. Much evidence indicates that the endothelial cells of newly forming blood-vessel capillaries are highly and selectively sensitive to very low doses of various chemotherapeutic drugs [9-12]. Some of the most interesting studies involve various microtubule inhibitors, such as vinblastine, paclitaxel and docetaxel.

To evaluate the activities of circulating endothelial progenitor cells (CEPs) or circulating endothelial cells (CECs) is a promising approach to determine the optimal low dose for a given metronomic chemotherapy regimen. CEP mobilization from the bone marrow into the peripheral circulation is strongly inhibited by low-dose cyclophosphamide, as is CEP viability [13]. Therefore, there could be a direct relationship between the relative efficacy of different doses used in metronomic chemotherapy and the ability of these doses to reduce levels of CEPs in the peripheral circulation.

Normalization of tumor vasculature by anti-angiogenic drugs is an emerging concept for improving the efficacy of cytotoxic chemotherapy and radiotherapy [14-17]. In 2001, R. K. Jain proposed that anti-angiogenic therapy could transiently normalize the tumor vasculature and thus improve the delivery and effectiveness of cytotoxic agents given during the normalization window [15,16]. This hypothesis offered a potential explanation for why a drug, such as bevacizumab, that is designed to destroy tumor blood vessels can augment the efficacy of chemotherapy and radiotherapy. Vascular normalization is also the key mechanism of action of the US Food and Drug Administration (FDA) approved anti-VEGF agent (Macugen) in the treatment of age-related wet macular degeneration (AMD) [18].

Current translational approaches are using small-molecule inhibitors or monoclonal antibodies that modulate various steps of angiogenesis processes, and several such agents have already received regulatory approval for the therapy of specific indications in cancer.

**Small-molecule inhibitors**

Several tyrosine kinase receptors play crucial roles in the angiogenesis of tumors, and may, therefore, serve as reasonable targets for chemotherapy (Table 1). The critical tyrosine kinase targets that have attracted the most scientific interest are VEGFR, FGFR, PDGFR and Tie-2 [19]. In the 1990s, a major step in targeted therapies was the appearance of imatinib (Gleevec, Glivec, STI571; Novartis), an agent that specifically targets Bcr-Abl, the tyrosine kinase fusion protein causing chronic myelogenous leukemia (CML) [20,21]. This drug was the first selective tyrosine kinase inhibitor (TKI) approved in 2001 for the treatment of CML [22]. In the recent years, several small-molecule drugs have been manipulated in clinical trials and even approved by FDA (Table 1). The multi-target approach has emerged as a
Table 1: Targets for small-molecule drugs in anti-angiogenic therapy

<table>
<thead>
<tr>
<th>Molecular targets</th>
<th>Small-molecule drugs</th>
<th>Current status</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth factor receptors</td>
<td>Gefitinib/Erissa®</td>
<td>FDA approved</td>
<td>[107]</td>
</tr>
<tr>
<td></td>
<td>Lapatinib/Tykerb®</td>
<td>FDA approved</td>
<td>[35]</td>
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<tr>
<td></td>
<td>Erlotinib/Tarceva®</td>
<td>FDA approved</td>
<td>[108]</td>
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<tr>
<td></td>
<td>Canertinib</td>
<td>Phase II</td>
<td>[37]</td>
</tr>
<tr>
<td>VEGFR</td>
<td>Vatalanib</td>
<td>Phase III</td>
<td>[109]</td>
</tr>
<tr>
<td>Multiple growth factor receptors</td>
<td>Imatinib/Glivec®</td>
<td>FDA approved</td>
<td>[110]</td>
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<tr>
<td></td>
<td>Sunitinib/Sutent®</td>
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<td>[111]</td>
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<tr>
<td></td>
<td>Sorafenib/Nexavar®</td>
<td>FDA approved</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>Pazopanib</td>
<td>Phase III</td>
<td>[33]</td>
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</tbody>
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new paradigm for the use of the new kinase inhibitors [23]. More specific single-target agents might not have significant effects on cancer complexity. In the multi-target approach, such agents are able to simultaneously target the tumor and supportive cells and thereby interact with the multi-molecular lesions driving tumor growth. With regard to the development of resistance caused by overexpression of key factors of signaling pathways, drug-efflux systems or signaling bypass owing to mutations, resistance is unlikely to occur with multi-targeted agents. Sunitinib malate (SU11248/Sutent; Pfizer), for example, is a multi-targeted TKI having anti-angiogenic and anti-tumor activities achieved through the selective inhibition of VEGFR, PDGFR, Kit, Flt3, Ret, and CSF1R [24-26]. Studies have demonstrated its definitive efficacy in advanced renal cell carcinoma (RCC) and in gastrointestinal stromal tumors refractory to imatinib and provided the basis for the current approval for specific indications [27].

Sorafenib is another multi-kinase inhibitor taken orally and recently approved for use in the treatment of metastatic RCC. It is currently undergoing investigation in locally advanced RCC and in other tumor types [28]. Recent findings indicate sorafenib, which was initially developed as an inhibitor of Raf kinase, has a broad activity against several tyrosine kinases, including angiogenic factors, VEGFR and PDGFR [29]. The FDA’s approval of sorafenib was established on the results of an international randomized placebo-controlled trial in the patients with inoperable hepatocellular carcinoma. The trial was suspended after an interim analysis showed patients receiving sorafenib to have extended survival, with those receiving sorafenib surviving a median of 10.7 months vs. placebo group who survived 7.9 months [30]. A separate analysis showed that tumors progressed more slowly in patients receiving sorafenib than in patients who had received placebo [30].

Pazopanib (GW786034) is a multi-targeted TKI against VEGFR-1, -2 and -3, PDGFR-α, PDGFR-β, and c-Kit. Preclinical evaluation has shown it to have excellent anti-angiogenic and anti-tumor activities and to have a synergistic effect when combined with chemotherapeutic drugs [31,32]. It was found to have significant anti-tumor effects in animal models of several tumors, accompanied with oral bioavailability [32]. A phase II clinical trial of pazopanib in untreated or cyto-kine/bevacizumab-pre-treated RCC has demonstrated promising anti-tumor activity as well as a favorable toxicity profile. A placebo-controlled phase III trial in untreated or cytokine-treated patients with RCC is currently underway [33].

Lapatinib is an oral dual TKI that targets EGFR and ErbB2/HER2, both frequently overexpressed in human cancer. Preclinical data have shown that the growth of tumor cells with these overexpressed receptors is inhibited by lapatinib both in vitro and in vivo [34]. In March 2007, lapatinib was approved by FDA to treat advanced or metas-

tatic breast cancer in the patients whose tumors overexpress HER2 and have received prior therapy including anthracycline, taxane and trastuzumab [35]. Canertinib (CI-1033), a pan-ErbB TKI, is a clinically promising drug active against all four members of the ErbB receptor tyrosine kinase family. In vitro studies of human cancer cell lines specify that canertinib causes potent and sustained inhibition of tyrosine kinase activity. This inhibition is highly selective for ErbB1, ErbB2, ErbB3, and ErbB4 without inhibiting tyrosine kinase activity of receptors such as PDGFR, bFGF and IGFR, even at high concentrations [36]. The open-label, randomized phase II trial evaluated canertinib in patients with advanced-stage non-small cell lung cancer (NSCLC) who experienced treatment failure after or were refractory to platinum-based chemotherapy. The one-year survival rate of these patients was nearly 30%. Exploratory analyses found prolonged survival in patients with a rash and in those with baseline tumor ErbB2 expression [37].

Monoclonal antibodies (MAbs)

The first chimeric therapeutic antibody, rituximab (Rituxan; Genentech/Biogen Idec), was generated in the late 1980s [38,39] and was approved in 1997 for the treatment of B-cell non-Hodgkin’s lymphoma. MAbs have been found to have great therapeutic potential for various pathological conditions, and their use has evolved into an active field in cancer research.

The use of MAbs is a promising approach to overcome the difficulties in differentiating tumor cells from normal ones because they could be designed to selectively target tumor cells and trigger various responses. These agents can either directly kill cells by bearing toxic material or instigating the destruction of cells in other ways, such as activating immune systems, blocking receptors, or exfoliating growth factors. From 1980 to 2005, a total of 206 unique therapeutic MAbs were studied in clinical trials by commercial companies worldwide for various cancer indications [40]. To date, twelve of these anti-cancer MAbs have been approved for sale in at least one country [40]. Several anti-angiogenic MAbs have been approved by FDA or studied in clinical trials (Table 2).

A humanized MAb directed against VEGF, bevacizumab, acts as a powerful angiogenic inhibitor. FDA approval of bevacizumab was first granted on the 26th of February 2004 after a successful phase III trial for treatment of metastatic colorectal cancer. In a randomized controlled trial of 813 patients with first-line metastatic colorectal cancer, the median duration of survival was 20.3 months in the patients who received bevacizumab accompanied with chemotherapy, compared to 15.6 months in those patients receiving chemotherapy alone [1]. FDA approval was then granted on the 11th of October 2006 for use of bevacizumab in combination with carboplatin and paclitaxel chemotherapy in NSCLC.
Table 2: Targets for monoclonal antibodies in anti-angiogenic therapy

<table>
<thead>
<tr>
<th>Molecular targets</th>
<th>Monoclonal antibodies</th>
<th>Current status</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>Bevacizumab</td>
<td>FDA approved</td>
<td>[41]</td>
</tr>
<tr>
<td>VEGFR-2</td>
<td>IMC-1C1</td>
<td>Phase I</td>
<td>[112]</td>
</tr>
<tr>
<td>VEGFR-3</td>
<td>mF4-31C1</td>
<td>Preclinical trial</td>
<td>[113]</td>
</tr>
<tr>
<td>Integrin α5β3</td>
<td>Vitanix</td>
<td>Phase I</td>
<td>[45][46]</td>
</tr>
</tbody>
</table>

Combination therapy has been reported to bring about a 25% improvement in survival compared with chemotherapy alone [41]. Several other clinical trials are currently under way studying the use of this MAB in the treatment of RCC, metastatic breast cancer, and cervical cancer [42]. Another approach for anti-angiogenesis is to inhibit the adhesive interactions between endothelial cells and the surrounding extracellular matrix (ECM). The integrin α5β3 is expressed at high levels in tumor vasculature and wound-healing tissues, but at extremely low levels in normal blood vessels [43]. The integrin α5β3 is also highly expressed on osteoclasts and tumor cells in various tissues. Therefore, impeding this integrin may have anti-tumor, anti-angiogenic, and anti-osteolytic effects [44]. Vitanix (MEDI-522), a humanized MAB antagonizing integrin α5β3, thus inhibits endothelial cell adhesion and proliferation in vitro and angiogenesis in vivo in skin and breast cancer [43]. Recently, McNeel and colleagues [45], in a study of 25 patients with metastatic solid tumors, reported Vitanix to have adverse effects, including minor infusion-related reactions (rigors, flushing, fever, injection site reactions, and tachycardia), low-grade gastrointestinal symptoms, and asymptomatic hypophosphatemia. Three of the patients with RCC experienced prolonged stable disease (varying from 34 weeks to 2 years), while no patients had complete or partial reversion. In spite that various experiments with Vitanix in vitro and in vivo have shown promising results, most phase I clinical trials have failed to demonstrate Vitanix to be efficacious in the treatment of human cancer [46]. Dass et al. pointed that further larger-scale studies would be needed to evaluate Vitanix’s potential in the treatment of cancer, particularly RCC [46].

**Toxicities induced by anti-angiogenic therapy**

There have been reasons to believe that anti-angiogenic drugs for clinical use may have fewer side effects and cause less drug resistance in cancer treatment. First, because endothelial cells have the genetic stability, anti-angiogenic agents would probably not active in causing resistance. Second, under normal physiological circumstances, more than 99% of endothelial cells are quiescent [47,48]. Third, the physiological angiogenesis is distinct from arteriogenesi-s and lymphangiogenesis and occurs in reproduction, development, wound repair, and activated growth factor pathways [49]. Therefore, it has been proposed that tumor-stimulated endothelial cells have a unique proliferating and migrating phenotype compared with quiescent endothelial cells, and that targeting this phenotype would be so specific that no major side effects could occur, except for during wound healing and the menstrual cycle when most endothelial cells are more active [50].

However, recent clinical experience has changed these expectations [51]. First of all, the generation of new blood vessels is a very complicated multi-step biological process and VEGF plays an important role in biologic effects including haematopoiesis, myelopoiesis and endothelial cell survival [52-54]. Therefore, anti-angiogenic therapy could cause several toxicities in these biologic effects. Furthermore, many of angiogenic inhibitors target multiple tyrosine kinases of several different pathways [55,56], and thus toxicities may not only arise from the inhibition of one pathway but also possibly from the concomitant inhibition of several pathways. Moreover, many of these biological agents are used or will be used in combination with other cytotoxic agents for treatment strategies [57,58]. It is not surprising that there are more toxicities in some studies using combination therapies involving some angiogenesis inhibitors than those using single agents [59,60]. Understanding the possible mechanisms that underlie toxicities induced by angiogenesis inhibitors may help us to develop more specific and potent anti-angiogenesis treatments.

The toxicities of angiogenesis inhibitors include bleeding, disturbed wound healing, thrombosis, hypertension, hypothyroidism and fatigue, proteinuria and edema, skin toxicity, leukopenia, lymphopenia, and immunomodulation. The schematic representation shown in Figure 2 illustrates the overall concept of toxicity induced by angiogenesis inhibitors.

**Bleeding and disturbed wound healing**

Anti-angiogenic therapy most probably disturbs the tight endothelial-cell-platelet interaction. Loss of vascular integrity will cause bleeding complications, gastrointestinal perforations and disturbed wound and ulcer healing. Bleeding complications have been reported in up to 44% of patients treated with bevacizumab [61] and have occurred in 26-60% of patients during treatment with anti-angiogenic TKIs [62].

Platelets can secrete VEGF in wound-healing areas, and the inhibition of platelet activation results in a significant decrease of VEGF in wounds [63]. During wound healing, platelets release their contents which are primarily growth factors. Interestingly, platelets express VEGFR, and VEGF enhances platelet activation. Wound healing problems increase from 3.4 to 13% when surgery is performed on colorectal cancer patients who are being treated with bevacizumab plus chemotherapy versus those treated with chemotherapy alone [64], and wound healing problems increased 0.5 to 1.3% during post-operative treatment of patients with a combination of bevacizumab and chemotherapy versus chemotherapy alone [65]. Besides, healing of gastrointestinal ulcers also depends on angiogenesis, and an adverse relationship between gastrointestinal perforations and anti-angiogenic antibody therapy has been found [65]. While the pathophysiology is unclear, it is known that VEGF is necessary for functioning of the intestinal villous capillaries.

**Thrombosis**

Endothelial cells play a major role in confining the coagulation reactions to a site of injury and preventing clot extension to areas where the endothelium is intact. To achieve this, many factors are produced and secreted by endothelial cells to prevent the activation and propagation of the coagulation cascade. VEGF stimulates coagulation by inducing tissue factor (TF) activity, vascular permeability and endothelial cell proliferation and migration at high concentrations. At low concentrations, VEGF is a survival and
Figure 2: The toxicities induced by anti-angiogenic therapy. Tyrosine kinase inhibitors (TKIs) and Avastin (bevacizumab) can block angiogenesis in tumors. These angiogenic inhibitors can induce apoptosis of endothelial cells and then cause tumor cell death. However, several toxicities can be induced using anti-angiogenic therapy: (a) bleeding complications and disturbed wound healing can occur as a result of anti-angiogenic therapy; (b) gastrointestinal perforations can be caused by the disturbance of intestinal villous capillaries during the anti-angiogenic therapy; (c) hypertension and a reduced left ventricular ejection fraction (LVEF) are associated with anti-angiogenic therapy and can also ultimately be life threatening; (d) the thyroid gland has many capillaries and anti-angiogenic TKIs can affect thyroid homeostasis and cause hypothyroidism and fatigue; (e) inhibition of VEGF may damage kidney, change the osmotic pressure and cause mild proteinuria and edema; (f) the inhibition of angiogenesis may involve in the growth factor signaling pathways of skin and cause skin toxicities; (g) leukopenia and lymphopenia could occur in patients with angiogenesis inhibitors; and (h) thrombotic events caused by endothelial cell damage have been observed in the patients treated with angiogenesis inhibitors.

maintenance factor for the endothelial cell lining. Blocking the VEGF pathway might induce apoptosis of the quiescent endothelial cells in vivo rather than inhibiting the angiogenic activity of VEGF [66]. Therefore, both proliferating endothelial cells and apoptotic endothelial cells can affect coagulation [67]. Thrombotic events have been observed in the patients treated with angiogenesis inhibitors, and bevacizumab has been found to primarily increase the risk for arterial thrombosis [61]. However, the incidence of drug-related thrombotic events seems low with small molecules of anti-angiogenic TKIs as monotherapy [59].

Hypertension

VEGF activates the endothelial cells to stimulate the production of nitric oxide synthase, producing nitric oxide. Nitric oxide is used to relax the surrounding smooth muscle of blood vessels. Thrombosis is defined as a pathological formation of a thrombus or clot in a vessel and causes an obstruction to the flow of blood. The treatment of cancer patients with angiogenesis inhibitors has been associated with hypertension and a reduced LVEF [68-70]. While the regulation of anti-hypertensive agents are quite effective in reducing the increase of blood pressure, bevacizumab- or anti-angiogenic TKI-induced hypertension might be life-threatening and cause damage to the eyes, brain, kidneys and lungs.

Hypothyroidism and fatigue

The thyroid gland has many capillaries and anti-angiogenic TKIs can affect thyroid homeostasis. In up to 36% of patients, an increase in thyroid-stimulating hormone and a decrease in the levels of the circulating thyroid hormones, indicative of hypothyroidism, have been observed after treatment with TKIs [71]. Disturbance in thyroid function might result in fatigue [68].

Proteinuria and edema

Proteinuria, the presence of an excess of proteins in the urine, may be an indicator of renal damage. Renal function is partly regulated by VEGF [72], and the inhibition of VEGF causes mild proteinuria [73,74]. Recently, Eremina et al. [75] have found that local reduction of VEGF within the kidney is sufficient to trigger the pathogenesis of thrombotic microangiopathy and glomerular injury in patients who have been treated with bevacizumab and this is probably due to direct targeting of VEGF by anti-angiogenic therapy. Because proteins are normally reabsorbed from urine, proteinuria is due to a decreased reabsorption or increased filtration. Moreover, when large amounts of proteins are lost in the urine, the balance of the osmotic pressure between the blood and the interstitium is disturbed. This will increase secretion of fluid into the interstitium or impair the removal of this fluid and cause edema.

Skin toxicity

The inhibition of angiogenesis can also cause severe skin toxicities. Growth factor signaling pathways are involved in the homeostasis of the skin. For example, hair depigmentation, hair loss and acral erythema are common during treatment with sunitinib or sorafenib [76]. The observation
that endothelial cells are affected in the skin suggests that these agents have a direct consequence on the biological activity in skin toxicity. This toxicity might be relative to the interaction between stromal and endothelial cells.

**Leukopenia, lymphopenia and immunomodulation**

VEGFRs are expressed by almost all haematopoietic cells and endothelial cell precursors [77,78]. Therefore, it is possible that inhibition of angiogenesis can cause leukopenia and lymphopenia, as well as thrombocytopenia. In addition, VEGF is known to inhibit the functional maturation of dendritic cells from progenitors [79]. Therefore, the inhibition of VEGF may have an effect on the immune system and induce immunomodulation.

**Vasculogenic mimicry**

In addition to toxicities induced by angiogenesis inhibitors, vasculogenic mimicry is another obstacle which needs to be overcome in anti-angiogenic therapy. Vasculogenic mimicry refers to the ability of cancer cells, including melanoma, lung, breast, prostatic and ovarian carcinoma, synoviosarcoma, thymidymosarcoma and phaeochromocytoma, to form ECM-rich vasculature-like networks, as shown by periodic acid schiff staining (PAS staining)[80]. There is a growing body of *in vivo* evidence that tumor cells can line channels, sinuses, lakes and vessel-like spaces, and come into contact with erythrocytes [81]. In addition, mosaic blood vessels have been observed in colon carcinomas and melanomas [82,83]. Morphological analysis has shown PAS-positive patterned networks to be found in aggressive melanomas and to be associated with poor clinical outcome [84,85]. In ovarian carcinomas, there has been an association found between vasculogenic mimicry and aggressive tumor cells and advanced-stage disease [86]. One follow-up blinded study has shown a strong clinical correlation between the presence of tumor cell-lined vasculature and advanced-stage disease and poor outcome [87]. Vasculogenic mimicry seems to involve dysregulation of the tumor cell-specific phenotype, expression of endothelium-associated genes, and the concomitant transdifferentiation of aggressive tumor cells into endothelial cells. For example, some aggressive melanoma cell lines and tumors overexpress VE-cadherin [88], whereas aggressive breast, ovarian and prostatic tumor cells overexpress CD31 [86,89,90].

Studies have indicated the presence of a fluid-conducting ECM meshwork in xenograft models of melanoma that corresponds to the PAS- and laminin-positive patterned network [83]. Immunohistochemical studies have shown that this fluid-conducting meshwork contains fibrinogen, indicating the presence of plasma surrounding the tumor cell-lined clusters of tumor cells [83]. The plasma, in addition to the erythrocytes that have been observed in many PAS- and laminin-positive loops and networks of tumors, is likely to be derived from leaky local tumor vessels undergoing remodeling.

The PAS- and laminin-rich, fluid-conducting meshwork could be an early survival mechanism for nutrient exchange and the release of fluid pressure. This meshwork could eventually be replaced by endothelial cells from nearby angiogenic vessels or from the bone marrow [91]. It has also been found that precancerous stem cells can serve as tumor vasculogenic progenitors [92]. An unexpected finding is the high-level expression by aggressive melanoma cells of VEGF-C. Interestingly, despite the fact that melanomas lack lymphatic vessels, these tumors have been reported to have an overexpression of VEGF-C [93]. Lymphangiogenesis often accompanies angiogenesis [94,95] and VEGF-C/FK-4 axis promotes angiogenesis, lymphangiogenesis, invasion and metastasis of cancer cells [96]. Ruoslahti and colleagues [97] have shown localization of lymphatic-vessel endothelial hyaluronan receptor 1 (LYVE1) in aggressive melanoma. These results raise the intriguing possibility that the fluid-conducting meshwork could “mimic” the lymphatic network.

Successful management of solid tumors could involve the identification of essential regulatory pathways and targeting stages in the signaling cascade of vasculogenic mimicry. There are both challenges and hopes found in the field of angiogenesis and anti-vascular targeting.

**Novel strategies for anti-angiogenic therapy**

There remain several hindrances to treatment of cancer by anti-angiogenic therapy, including low selective toxicity of anti-cancer drugs, high tumor interstitial fluid pressure (IFP)-impaired transport of drug, vasculogenic mimicry in solid tumors, toxicities induced by anti-angiogenic therapy, and drug resistance. Novel strategies for overcoming these problems are urgently necessary.

Tumor blood vessels present certain unique characteristics that are not observed in normal tissues, including excessive angiogenesis, lack of mature vascular architecture, impaired lymphatic drainage, and increased expression of permeability mediators on the cell surface [98]. These characteristics may be used in development of anti-angiogenic target therapy for cancer and may help locate the site of tumor in which a nanoscale drug delivery system might be concentrated. The particular strength of drug delivery systems is their ability to alter the pharmacokinetics and the biodistribution of their associated therapeutic agents [99]. When a drug is associated with a carrier, the drug clearance decreases (the half-life increases), the volume of distribution decreases, and the area under the time-versus-concentration curve increases [100,101]. The diameter of liposome is approximately 65-75 nm [102-104]. However, because tumor vessels lack tight junctions between adjacent vasculature endothelial cells, the size of the gaps between the cells that line tumor blood vessels has been estimated to be 100-500 nm [105], which is large enough to allow the extravasation of most liposomes from the vessel into the tumor interstitial space through these pores. Liposomes are not capable of extravasation from the blood stream into normal tissues that have tight junctions between capillary endothelial cells, e.g., heart. Therefore, the level of drug reaching at sensitive tissues is reduced and this is the mechanism can help reduce side effects of liposomal drugs compared to free drugs. The preferential accumulation of liposomal anti-cancer drugs in solid tumors is referred to as “passive targeting” [106]. While clinically useful anti-tumor activity has been achieved using PEGylated liposomes, higher and more selective anti-cancer activity of liposomal anti-cancer drugs is possible by ligand-mediated targeting liposomes. This involves the coupling of targeting moieties to the surface of liposomes to make targeting liposomes. This next generation of drug delivery system is referred to as “active targeting”. The ligands promote the selective binding of the liposomes to tumor-associated antigens and facilitate the delivery of the drug-liposome packages to the cellular site of drug action. Therefore, efforts are being made to enhance the site-specific actions of targeting liposomes by combining them with the ligands targeted against tumor cells or tumor vasculature [102-104,108].

Using *in vivo* phage display, Lee et al. [102] have identified a novel peptide SPS-52 which specifically binds to tumor blood vessels. Several selected phage clones display the consensus motif Pro-Ser-Pro and this motif is crucial for peptide binding to tumor neovascularature. SPS-52 peptides
also bind blood vessels of human lung cancer surgical specimens. Furthermore, this targeting phase has been shown to home to tumor tissues from eight different types of human tumor xenografts by in vivo homing assay [102]. The SP5-52-targeted liposomal doxorubicin has been found to have an enhanced anti-tumor effect and offer significant clinical potential in a targeted drug delivery system [102].

Tumors treated with targeting liposomes demonstrate a marked decrease in vessel density, high level of cancer cell death and inhibited tumor growth compared to those treated with non-targeting liposomes [102,104]. Discovery of ligands that target cancer cells (including cancer stem cells) and tumor blood vessels, and development of ligand-targeted therapy will improve the therapeutic efficacy and reduce side effects. Unlike other forms of therapy, this approach helps effectively attack cancer and minimize the side effects [102-104,106].

Challenge and perspective

Tumor angiogenesis has been considered as an especially useful target for therapy and the effect of anti-angiogenic therapy has improved the therapeutic index. At present, angiogenesis inhibitors have been shown to prolong progression-free survival but only have a small effect on overall survival in patients with cancer. The inhibition of angiogenesis has given us a straightforward means of treating cancer, though many anti-angiogenic agents have side effects. Therefore, understanding the biological mechanisms underlying these toxicities may help optimize such strategies.

The development of targeted therapies against cancer, with improved discrimination between tumor cells and non-malignant counterparts, is one of the major goals of current anti-cancer research. Peptides and antibodies that bind to specific markers of tumor vasculature can also be used to deliver cytotoxic or diagnostic agents to tumors. By coupling peptides to the polyethylene glycol termini of sterically stabilized liposomes, we have taken the first step toward optimizing liposome-based delivery to improve the next generation of vasculature-targeting therapeutic agents [102]. This tumor site-specific target therapy may overcome the adverse effect caused by systemic therapy using anti-angiogenic drugs and increase the therapeutic index [102-104]. Furthermore, identification of target molecules expressed on vasculoagenic mimicry tumor vessels and development of ligand-targeted therapy may help overcome drug resistance generated by anti-angiogenic agents. The lymphatic vessels in tumors have not yet been subjected to specific targeting. An important incentive to develop methods for targeting tumor lymphatics is that destroying these vessels could eliminate an important route of tumor metastasis. Finally, further research is needed to determine whether the putative tumor cell-derived vascular channels might constitute other useful targets for new therapies.

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References


