Antiangiogenic Strategies on Defense: On the Possibility of Blocking Rebounds by the Tumor Vasculature after Chemotherapy

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Abstract

Rapid or accelerated tumor cell repopulation after significant tumor cell killing induced by various cytotoxic agents often compromises the expected therapeutic benefit of such tumor responses. Here, we discuss the concept that tumor cell repopulation after certain cytotoxic therapies, using vascular disrupting agents as an example, may be aided by a reactive, systemic host response involving the mobilization of bone marrow–derived circulating cells, including endothelial progenitor cells, which subsequently home to the vasculature of treated tumors and promote tumor neovascularization. These vasculogenic "rebounds" can be blocked, at least in some cases, by treatment with an antiangiogenic drug. There is limited preliminary evidence that maximum tolerated dose chemotherapy causes a similar effect. This could constitute one way by which antiangiogenic therapy could increase the efficacy of conventional cytotoxic chemotherapy regimens; it also raises the specter of new molecular targets for systemic cancer therapies which are involved in therapy-induced bone marrow–derived cell mobilization, homing to tumors, and tumor retention. [Cancer Res 2007;67(15):7055–8]

Introduction

The recent clinical success of a number of antiangiogenic drugs (1–3) has raised a somewhat ironic question: how do they actually work to cause a clinical benefit? The common perception is that they "starve" tumors of the oxygen and nutrients necessary for survival and relentless growth. If so, it would follow that antiangiogenic drug therapy on its own should have a significant clinical benefit, including prolonging survival even in the setting of advanced metastatic disease. This might turn out to be the case with "multitargeting" small molecule receptor tyrosine kinase inhibitors such as sorafenib or sunitinib in the treatment of renal cell carcinoma (3, 4) and other cancers such as hepatocellular carcinoma. However, given the multiple kinases targeted by such drugs, there is the possibility that some of the therapeutic benefits are caused by mechanisms independent of antiangiogenesis. In the case of specific antibody-targeting drugs, such as bevacizumab, the humanized monoclonal antivascular endothelial growth factor (VEGF) antibody, clinical benefit in some randomized phase III trials is observed when it is combined with chemotherapy, e.g., 5-fluorouracil/leucovorin and irinotecan for metastatic colorectal cancer (2) or paclitaxel and carboplatin for non–small cell lung cancer (1). Even then, the survival benefits are fairly modest (in the range of months). The bevacizumab results have stimulated discussion and debate about how this drug increases the efficacy of chemotherapy (5, 6).

Diverse Theories to Account for Antiangiogenic Drug–Mediated Increases in Chemotherapy Efficacy

Perhaps the best known proposed mechanism is the "vessel normalization" hypothesis of Jain and colleagues (6). Essentially, this refers to the effect of an antiangiogenic agent to transiently reverse some of the characteristics of the chaotic and dysfunctional tumor vasculature, and hence, certain features of the tumor microenvironment associated with such abnormal vasculature which may act to reduce the efficacy of chemotherapy. These features include high interstitial fluid pressures in tumors caused by the leakage of high molecular weight plasma proteins from the hyperpermeable tumor vasculature (which can be caused by the strong vascular permeability function of VEGF), sluggish/suboptimal blood flow of many tumor vessels which would increase tumor hypoxia, and thus, reduce cell proliferation of tumor cells in such hypoxic regions. In addition to causing some pruning of the immature vasculature, antiangiogenic agents can cause normalization/maturation of some of the remaining tumor vasculature, thus reducing high intratumoral interstitial fluid pressures and transiently reducing tumor hypoxia at the same time, and thus, increasing tumor cell proliferation (7). If chemotherapy is administered during the periods of antiangiogenic drug–induced tumor vascular normalization, there is the possibility of not only increased intratumoral drug delivery within tumors, but also an increase in the number of proliferating tumor cells that would be expected to be more sensitive to chemotherapy (7). It is of interest that this could also possibly lead not only to an increase in generalized tumor cell killing, but also to enhanced targeting of the tumor stem cell or the stem cell–like minor subpopulation. This may be particularly relevant in situations in which tumor stem cells are thought to reside in a vascular niche (8). Disruption of the vascular niche by an antiangiogenic drug could compromise tumor stem cell survival, and chemotherapy could conceivably enhance the susceptibility of the compromised stem cell subpopulation to chemotherapy (9). This proposed chemotherapy effect might be enhanced by vascular normalization.

An alternative, although not necessarily mutually exclusive, theory is that the tumor cell repopulation, which can often be quite robust (10, 11) following cytotoxic therapy, can be slowed as a result of the presence of an antiangiogenic agent during the drug-free break periods between successive cycles of chemotherapy drug administration (10). This is based on the reasonable assumption that surviving and repopulating/dividing tumor cells would require angiogenesis for optimal tumor cell survival and growth conditions. We have been investigating a new aspect of this hypothesis, i.e., the rate of tumor cell repopulation, which is known to become

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increasingly rapid with successive cycles of cytotoxic (e.g. radiation) therapy (11), can be inadvertently assisted by host-mediated systemic proangiogenic and vasculogenic processes (12). Based on our findings, following administration of certain cytotoxic agents, there can be a rapid (e.g., within hours) and marked mobilization of bone marrow–derived circulating endothelial progenitor cells (CEP), which subsequently enter the peripheral blood circulation as can be seen in Fig. 1*A*. These cells home to treated tumor sites, and in some fashion, promote and amplify the process of tumor angiogenesis (12). This was the subject of a recent report from our group, in collaboration with several others, in which this paradigm was studied using vascular disrupting agents (VDA) as a model of an anticancer "cytotoxic" drug (12).

Biology of VDAs

VDAs represent one of the two major classes of vascular targeting agents (13). In contrast to antiangiogenic drugs such as anti-VEGF or anti–VEGF receptor-2 (VEGFR-2) antibodies, which act primarily by preventing new blood vessel formation as well as targeting recently formed immature blood vessels, VDAs target the existing, more established but abnormal vasculature in tumors by a process likely involving the induction of endothelial cell apoptosis (14). This can lead to a rapid shutdown in tumor blood flow (within hours), and subsequently, massive intratumoral cell death (14). However, a rim of viable tumor tissue remains from which tumors can rapidly repopulate (13, 14), accompanied by robust angiogenesis. Consequently, investigators such as Siemann and colleagues have proposed, and subsequently showed, that the combination of a VEGF or VEGFR-2 targeting antiangiogenic drug with a VDA such as combretastatin could have complementary antitumor effects and therefore bring about an overall improved therapeutic benefit (15).

The ability of the surviving tumor cells to rapidly repopulate tumors after VDA treatment is conceptually similar to tumor cell repopulation after an initially effective cytotoxic chemotherapy or radiation treatment regimen. In this regard, using VDAs, we recently reported that the ability of tumors to repopulate after therapy with such a drug was significantly facilitated by an acute outflow of cells from the bone marrow, which then home through the blood circulation to the remaining viable tumor rim; some of

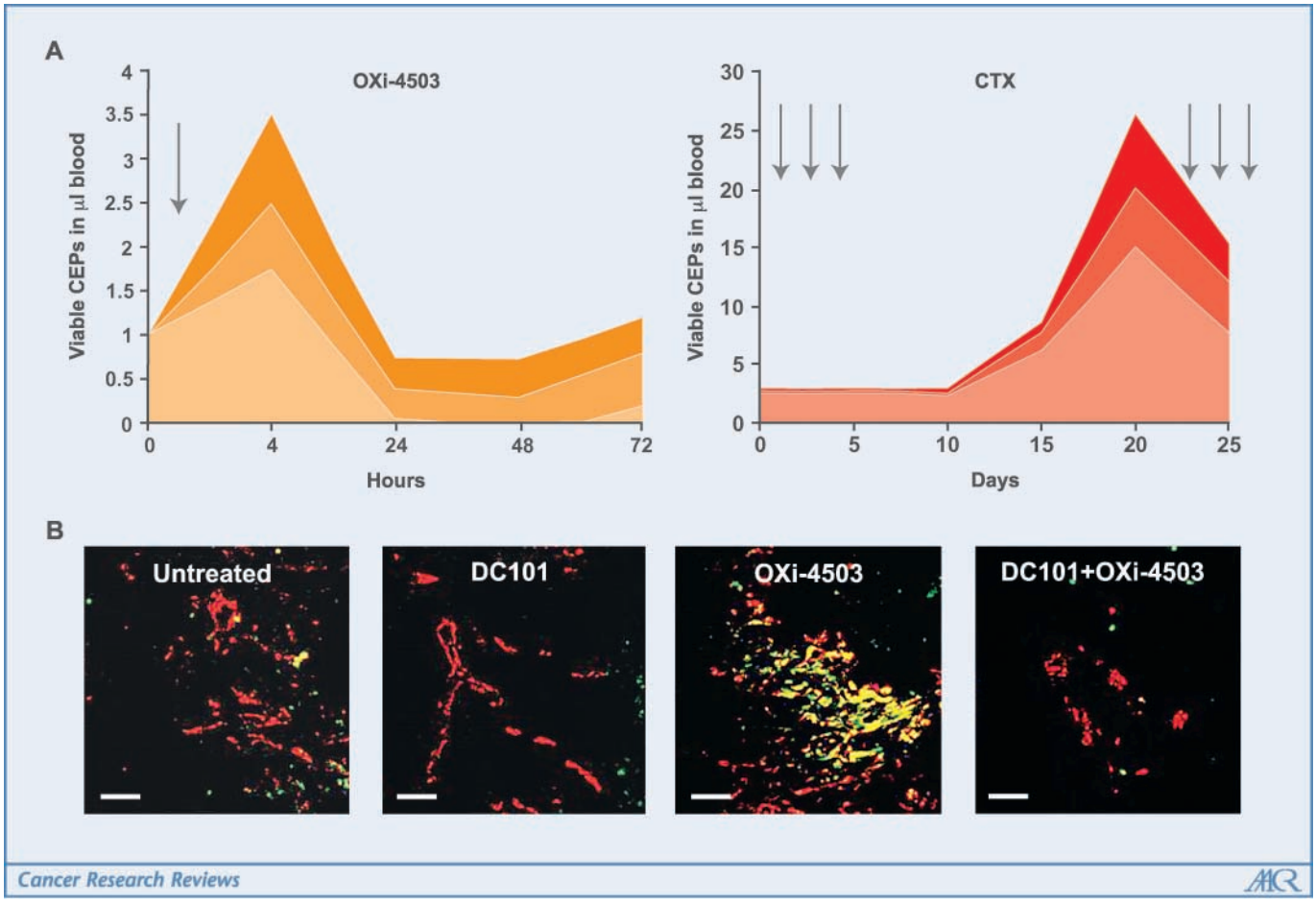

Figure 1. CEPs are mobilized from the bone marrow to peripheral blood, and subsequently home to the viable tumor rim remaining after treatment with a VDA. *A,* mice treated with a cytotoxic drug, either a VDA (OXi-4503) or cyclophosphamide (CTX), were bled from the retro-orbital sinus at several time points. CEPs were evaluated using four-color flow cytometry. A "spike" in CEPs in the peripheral blood was observed 4 h after OXi-4503 treatment, or several days after CTX treatment which involves 3 injections over a 6-d cycle of treatment. *B,* sections from Lewis lung carcinoma tumors grown in C57Bl/6 that were previously lethally irradiated and transplanted with GFP-positive–tagged bone marrow cells (*green*), were stained for VEGFR-2 (*red*). A massive invasion and infiltration of GFP-positive bone marrow–derived cells into the viable tumor rim site was observed within days after treatment with OXi-4503, most of which were also VEGFR-2–positive, as indicated by the colocalization of GFP and VEGFR-2. Treatment with DC101, a monoclonal antibody to mouse VEGFR-2, before VDA treatment, however, prevented colonization of most of the VEGFR-2–positive/GFP-positive cells at the tumor site normally induced by VDA treatment [reproduced from Shaked et al. (12) with permission from the publishers]. Scale bar: 50 μm.

these cells subsequently incorporate into the lumens of the tumor vasculature. That some of these cells were endothelial progenitor cells which differentiate into mature endothelial cells after incorporation into the lumen—a process known as adult or postnatal vasculogenesis—was implicated by several experimental approaches. One was to evaluate the effects of VDA treatment on tumors transplanted into recipient mutant mice, which are unable to mobilize endothelial progenitor cells, i.e., $Id1^{-/+}$ $Id3^{-/-}$ mice (16). In such a host background, the effect of the VDA in inducing tumor cell necrosis was significantly enhanced. Similarly, we undertook a pharmacologic "knockout" experiment, i.e., administered a targeted antiangiogenic drug, such as an antibody to VEGFR-2, 1 day prior to the administration of the VDA, combretastatin, or a second-generation derivative called OXi-4503 (12), both of which are microtubule-inhibiting agents. The spike in peripheral blood CEPs, along with their ability to home in large numbers to the viable rim of VDA-treated tumors, was almost completely blocked by the antiangiogenic agent. An example of this is shown in Fig. 1*B*. In this experiment, C57Bl/c mice were lethally irradiated and reconstituted with green fluorescent protein (GFP)–tagged bone marrow cells. The mice were allowed to recover and then used as recipients for s.c. injection of Lewis lung carcinoma cells. Once the tumors reached a certain size (e.g., 500 mm^3), the mice were treated with an antiangiogenic drug, DC101 (an anti–VEGFR-2 antibody) or a VDA (OXi-4503). The tumors from VDA-treated mice clearly "light up", i.e., contain large numbers of infiltrating GFP-positive bone marrow cells (which we sometimes call the "Christmas tree" effect), some of which were found to incorporate into the lumens of tumor-associated blood vessels, and thus seem to be endothelial progenitor cells. In contrast, the tumors of untreated control mice contained very few GFP-positive cells, as was the case for DC101-treated tumors. Importantly, the strong GFP-positive signal in the VDA-treated tumors could be almost entirely obliterated by prior treatment with the DC101 antiangiogenic antibody. In other words, the Christmas tree "lights" were turned off by treatment with DC101. This may also suggest that most of the bone marrow–derived (GFP-positive) cells invading the tumor site after treatment were VEGFR-2–positive (Fig. 1*B*). Moreover, this treatment combination was accompanied by enhanced overall antiangiogenesis, enhanced tumor hypoxia, enhanced levels of tumor necrosis, decreased tumor blood flow, and enhanced overall antitumor growth effect (12).

In our study, we also noted that many of the GFP-positive bone marrow cells were located immediately adjacent to blood vessels. Some of these cells may stimulate angiogenesis by paracrine mechanisms such as VEGF secretion. In this regard, a variety of proangiogenic cell types having hybrid "vascular leukocyte" properties have been described recently (17), which express the CD45 hematopoietic marker as well as various endothelial cell and myeloid/monocytic markers (18–20). These cell populations are not endothelial progenitor cells but nevertheless can be mobilized from the bone marrow. It will be of interest to determine whether any of these cell types might also be mobilized by cytotoxic anticancer therapies, and home to the viable tumor tissue that remains after such therapy.

Do the Results Have Implications for Other Cytotoxic Anticancer Drugs Such as Chemotherapeutics?

There are a number of potential implications for these results. First, they may not only apply to cytotoxic VDAs, but also to conventional cytotoxic chemotherapy drugs administered at or near maximum tolerated doses. Although this is very speculative, and VDAs are quite different in nature from chemotherapeutic drugs, it should be noted that we previously reported the ability of maximum tolerated dose chemotherapy, using cyclophosphamide, to cause a rapid rebound of CEPs, following an initial decline, soon after the completion of a cycle of therapy, as shown in Fig. 1*A* (21). We did not, however, evaluate whether such CEPs homed to treated tumors and subsequently contributed to tumor angiogenesis and regrowth, but this would not seem to be an unreasonable possibility. Of note, there is some limited evidence to suggest that similar CEP vasculogenic rebounds could be detected in patients receiving conventional cytotoxic chemotherapy (22). It will therefore be of considerable interest to evaluate the effects of different chemotherapy drugs, and classes of such drugs, for their relative effects in inducing mobilization and intratumoral homing of bone marrow–derived cell populations which have the potential to stimulate tumor neovascularization. If some chemotherapy drugs are found to have an effect that is similar in nature to that described in our VDA studies, it will naturally be important to assess whether combination therapy with an antiangiogenic drug such as bevacizumab or a small molecule antiangiogenic receptor tyrosine kinase inhibitor can block such proangiogenic/provasculogenic (and hence potentially tumor growth–promoting) rebounds.

Also of interest is the fact that cytotoxic chemotherapy is sometimes accompanied by hematopoietic growth factor support using recombinant granulocyte colony-stimulating factor (G-CSF) to accelerate recovery from myelosuppression by mobilizing hematopoietic stem cell progenitors from the bone marrow. However, there is evidence that G-CSF can also mobilize CEPs (23, 24). Thus, cytotoxic chemotherapy–induced mobilization of CEPs might be amplified by coadministration of G-CSF. If so, the use of antiangiogenic drugs in such treatment settings, e.g., "dose dense" chemotherapy with G-CSF for adjuvant therapy of early stage breast cancer (25), would seem logical.

A second implication of our results concerns the continuing controversy about the contribution of bone marrow–derived endothelial progenitor cells to tumor angiogenesis. The controversy stems in significant part from conflicting findings regarding the relative contribution of endothelial progenitor cells to new blood vessel formation in tumors (24). Some studies have reported high proportions of such cells in newly forming blood vessels (e.g., 20–50%), but many others report much lower numbers, often 5% or less (24, 26). The results in Fig. 1*B* are consistent with the view that in many tumors, the relative contribution of such cells may indeed be minor, although this can change with such factors as tumor stage, size, degree of hypoxia, and other factors (24). However, as also shown in Fig. 1*B*, this contribution can suddenly and dramatically increase after a cytotoxic therapy. In this regard, it is important to note that all previously published studies dealing with the detection and enumeration of CEPs in tumor-associated blood vessels dealt with untreated tumors, or tumors that had been treated a long time before their removal and analysis (27). Acute mobilization of CEPs might be analogous to instances of ischemic cardiovascular disease in which there can be a sudden and marked increase of endothelial progenitor cells from the bone marrow, which home to sites of damaged blood vessels, ostensibly to repair such damage and regenerate new vessels (28). Such acute mobilization may be a part of an "SOS" host response, which, unfortunately, may also occur with respect to damaged vessels

in tumors, caused not only by VDA, but possibly cytotoxic chemotherapy regimens (29).

Finally, a third implication of the results is that they may be exploited for uncovering new targets for various antiangiogenic or other types of drug. For example, the molecular mechanisms responsible for the acute mobilization, homing, and retention within tumors of bone marrow–derived cell populations could clearly implicate new targets for anticancer agents (30), some of which may already be developed, or which could be developed in the future.

In summary, there are multiple possible mechanisms to explain how an antiangiogenic drug such as bevacizumab, and likely other antiangiogenic agents, can amplify the efficacy of chemotherapy. We have focused our comments on one particular mechanism. It is clearly possible that different mechanisms may predominate in different circumstances, or may even work simultaneously. For example, an antiangiogenic drug may simultaneously enhance chemotherapy efficacy by mechanisms associated with vascular normalization while at the same time blunting vasculogenic/angiogenic systemic rebounds.

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