Targeting angiogenesis with monoclonal antibodies

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Introduction

Anti-angiogenesis has been a major area of cancer research over the last 15 years and the recent entry of the first anti-angiogenic agents into phase III randomized trial has generated intense interest [1, 2]. The purpose of this article is to summarize where we currently stand in the use of monoclonal antibodies as anti-angiogenic agents. The anti-vascular endothelial growth factor antibody bevacizumab is rapidly becoming the most studied agent of this type.

Antibodies that target angiogenesis directly: vascular endothelial growth factor and bevacizumab

Vascular endothelial growth factor and tumor angiogenesis

First isolated in the 1980s, vascular endothelial growth factor (VEGF) (also known as vascular permeability factor) has emerged as a key player in tumor angiogenesis [3]. A dimeric protein expressed from a range of mRNA splice variants, its restricted specificity as a growth and migratory factor for endothelium are unusual. VEGF expression is induced by hypoxia and glucose deprivation and it is almost universally expressed in tumors. VEGF was shown to have potent angiogenic activity in vivo and several early studies showed that transfection of VEGF into tumor cell lines conferred a growth advantage on xenografts in vivo but not in vitro [4, 5]. This effect was attributed to enhanced tumor angiogenesis conferring an in vivo growth advantage. While other members of a VEGF ‘family’ exist, none show the widespread tumor expression and angiogenic potency of what is now referred to as VEGF-A. The first indication that VEGF may be a genuine anti-tumor target came from the 1992 study by Kim et al. [6] showing that monoclonal antibodies to VEGF slowed the growth of xenografted tumors in nude mice. This observation prompted Genentech Inc. to embark on a major program to develop anti-VEGF antibodies as a therapeutic agent for the treatment of cancer. The program lasted over ten years and showed that monoclonal antibodies to VEGF-A in combination with chemotherapy improved overall survival in colorectal and lung cancer patients and progression-free survival in breast cancer patients. In contrast, bevacizumab in combination with chemotherapy failed to increase survival in patients with previously treated and refractory metastatic breast cancer. Some of these results will now be described in more detail.

The first breakthrough for anti-angiogenic therapy in the clinic came from a randomized phase III trial which showed a 4.7-month increase in overall survival of patients with previously untreated metastatic colorectal cancer when bevacizumab was administered in combination with chemotherapy (irinotecan/5-fluorouracil/leucovorin) versus chemotherapy alone [8]. As a result of this study bevacizumab was approved by the Food and Drug Administration (FDA) for first-line therapy in the treatment of colorectal cancer in the US.

Bevacizumab has shown efficacy in three other randomized trials. Thus, bevacizumab in combination with paclitaxel in the treatment of chemotherapy-naïve recurrent or metastatic breast cancer showed improved progression-free survival [9]. Patients with previously treated advanced colorectal cancer showed a 2.1-month increase in overall survival when receiving bevacizumab in combination with second-line therapy oxaliplatin/5-fluorouracil/leucovorin regimen known as FOLFOX 4 versus FOLFOX 4 alone [10]. Finally, a 2.3-month increase in median survival was seen in previously untreated advanced non-squamous and non-small-cell lung cancer when bevacizumab was given in combination with paclitaxel and carboplatin versus chemotherapy alone [11].

These trials clearly show that bevacizumab can increase overall and/or progression-free survival when used in combination with chemotherapy, although the increased survival is admittedly modest and the conclusions are by some considered overstated [12]. The side effects of bevacizumab are either mild or rare and the safety of its use is good. For example, the most common side effect is hypertension that is in most patients manageable with medication. Rarely occurring but serious effects include thrombo-embolic events, gastrointestinal perforations and an increase from 0.5% to 2.2% in congestive heart failure in patients previously receiving anthracyclines [13].

In the light of bevacizumab’s efficacy, the question arises as to what bevacizumab is doing to the tumor. The original idea was that an anti-angiogenic would block growth of new vessels.
Antibodies approved for the treatment of cancer incorporating an anti-angiogenic component

<table>
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...to the tumor and so starve it of oxygen and nutrients. Indeed, one phase I study has shown that a single dose of bevacizumab in colorectal patients can prune tumor vessels and reduce tumor vascular density by up to 50%.

Despite this, no effect on survival has yet been seen in man on treatment with bevacizumab alone. Increased survival is only seen when bevacizumab is delivered in combination with chemotherapy. This has led researchers to look for other reasons for the clinical efficacy of bevacizumab.

One currently popular idea is that of ‘normalization of the vasculature’, an idea originally proposed in the 1970s [14]. The argument starts with the knowledge that tumor vessels are poorly formed, being tortuous, lacking supporting cells such as vascular smooth muscle and pericytes and showing poor blood flow [15, 16]. These immature vessels are known to form in the presence of excess VEGF. Removal of the excess VEGF by administration of bevacizumab induces maturation of the vessels mature and increases blood flow to the tumor. The increased blood flow permits oxygen and cytotoxic drug to flow into the tumor where the oxygen promotes an increase in proliferating tumor cells that are then killed by the cytotoxic drug targeting proliferating cells.

While strong supporting evidence for tumor normalisation is still lacking, there exists inferential evidence [17]. Amongst the latter is the observation that bevacizumab alone increases cancer-cell proliferation in colorectal cancer of some patients, suggesting increased perfusion of oxygen and nutrients to the tumor. In addition, there is no increase in fluorodeoxy-glucose in colorectal tumors of patients receiving bevacizumab despite a 50% reduction in vascular density, suggesting an increase in blood flow. Oxygen is a radiosensitizer and its proposed increase in delivery to the tumor would predict efficacy if bevacizumab would be used in combination with radiotherapy. Such trials are in progress.

More convincing evidence for vascular normalization would be provided by structural studies such as vascular casts before and after bevacizumab administration and dynamic measurements of tumor blood flow.

antibodies that target oncogenic pathways promoting angiogenesis or pivotal adhesion molecules

oncogenic pathways

VEGF expression lies downstream of several transforming oncogenic pathways with the result that antibodies that abrogate those pathways exert an indirect anti-angiogenic effect. Examples include the epidermal growth factor/HER-2 targeting antibodies cetuximab (Erbitux®) (Imclone, New York) and trastuzumab (Herceptin®) (Genentech, San Francisco). Both antibodies show increased overall survival when used in combination with chemotherapy in clinical trials and are approved for use in man in both Europe and the US. Thus, in a phase III trial of trastuzumab in combination with chemotherapy (anthracycline, cyclophosphamide or paclitaxel) versus chemotherapy alone, the combination gave significant overall survival benefit in Her-2 positive metastatic breast cancer patients [18]. More recently, two phase III trials have shown that breast cancer patients with Her-2 positive tumors receiving trastuzumab in combination with doxorubicin, cyclophosphamide and paclitaxel showed a 52% decrease in disease recurrence compared with patients treated with chemotherapy alone [19, 20].

That anti-angiogenesis is probably involved here was shown in a pre-clinical study in which trastuzumab decreased the expression of several angiogenic factors, including VEGF, by cancer cells while at the same time increasing expression of the anti-angiogenic thrombospondin-1 [21]. These expression changes were accompanied by vascular changes within the tumor, including a reduction in vascular permeability, vessel diameter and vascular volume. Such changes are consistent with a normalization of the tumor vasculature as has been proposed to occur with anti-VEGF antibodies.

adhesion molecules

Arguably the most studied anti-angiogenic antibody to an adhesion molecule is an anti αβ3 integrin antibody. This antibody in its humanized form is known as Vitaxin. Studies with the antibody are based on an original observation that the αβ3 integrin is highly expressed on angiogenic (newly forming) blood vessels and that an antibody to this integrin showed potent anti-angiogenic activity in a range of assays [22]. There is currently an ongoing phase I clinical trial of vitaxin in patients with advanced malignant melanoma.

why does bevacizumab not show greater efficacy?

The most likely reason that bevacizumab fails to show greater efficacy is the degenerate nature of the tumor angiogenic stimulus. For example, in 1997 Relf et al. showed that primary breast cancers produce as many as seven different angiogenic factors [23]. If all tumors are so diverse in their angiogenic factor production it is surprising that bevacizumab shows efficacy at all. It also illustrates the difficulty in targeting individual angiogenic factors. Although a great many angiogenic factors have been identified, expression of some is more widespread in some tumors than others. Particularly widely expressed along with VEGF are interleukin-8, adrenomedullin and the angiogenic enzyme thymidine phosphorylase [24, 25]. It is conceivable that cocktails of antibodies and thymidine phosphorylase inhibitors could see clinical use in the long-term future.
the need for new tumor vascular targeted therapies beyond bevacizumab

While we can, in principle, consider multiple angiogenic factor blocking strategies, the use of combination therapy and the difficulty in identifying key factors makes this approach less attractive. Rather, a complementary approach utilizing the unique properties of the tumor vasculature would offer an exciting alternative. One such approach is tumor vascular targeting [26]. The basic idea is to target toxic antibody-conjugates to antigens expressed only on or around the tumor vessels and not on vessels in normal tissues. Selective destruction of the tumor vasculature has been shown to be an effective strategy to eradicate tumors in animal models [27]. Although Thorpe and co-workers provided this proof of principle over ten years ago, it has proven difficult to identify suitable targets in human tumors. In recent years this has changed with the identification of tumor stromal and endothelial markers such as the B-(oncofetal) domain of fibronectin and the endothelial specific roundabout gene Robo4. Vascular targeting, like anti-angiogenesis, is at an exciting phase with the entry of the first anti-fibronectin B-domain antibodies into phase I clinical trial.

closing comments

Antibodies are finally finding use in the therapy of solid tumors and anti-angiogenic antibodies are prominent amongst them. Nevertheless there remain problems. Foremost amongst these is cost, especially when balanced against modest efficacy. Oral delivery of anti-angiogenics intended for long term (and possibly prophylactic) such as the VEGF receptor tyrosine kinase inhibitors will in all probability in time replace antibodies. Nevertheless, the first-generation anti-angiogenics have show proven efficacy in a range of cancers and there clearly exists much room for improvement.

acknowledgements

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references