

Angiogenesis: Novel and Basic Science Insights and Human Therapy - Keystone Symposium

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Introduction

This meeting, chaired by Napoleone Ferrara (Genentech Inc, USA), Shahin Rafii (Cornell University Medical College, USA) and Elisabetta Dejana (Istituto di Ricerche Farmacologiche Mario Negri, Italy), attracted over 300 scientists from academia, industry and clinical centers. In a total of nine sessions, the 42 speakers and 135 posters described the latest aspects of angiogenesis research. There was an excellent mix of basic science and data from preclinical and clinical trials. The meeting provided an encouraging atmosphere for interaction, an exchange of ideas and initiation of collaborative efforts. Several novel potential therapeutic targets were discussed, along with ongoing trials. The update on the colon and breast cancer phase III trials of bevacizumab (Avastin; F Hoffmann-La Roche Ltd/Genentech Inc) was a highlight of the meeting. This report will particularly discuss those talks concerning biotechnology. Emphasis has been placed on new targets and developments in basic science that are applicable to therapeutic approaches.

Ongoing clinical trials of VEGF inhibitors

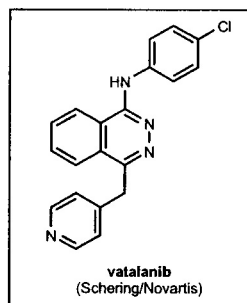
Bevacizumab

The success of bevacizumab, communicated in May 2003 at the American Society for Clinical Oncology (ASCO) meeting, has continued. William Novotny (Genentech Inc, USA) presented an update on the clinical trials of this recombinant, humanized, monoclonal anti-vascular endothelial growth factor (VEGF) antibody. A randomized trial in colorectal cancer patients included the following arms: (i) group A, bolus irinotecan, 5-fluorouracil, leucovorin (IFL) chemotherapy (n = 110); (ii) group B, bolus IFL plus placebo (n = 412); and (iii) group C, bolus IFL plus bevacizumab (5 mg/kg every 2 weeks; n = 403). Median survival was increased by 5 months (the difference between groups B and C) with bevacizumab and a 34% reduction in the number of deaths was observed. Similar benefits were noted for progression-free survival. The only side effect noted was grade 3 hypertension. Dr Novotny stated that this is an interesting outcome that suggests a homeostatic role for VEGF in the regulation of blood pressure. No grade 4 hypertension cases were noted but occasional gastrointestinal perforations were observed (six of 393 adverse events noted). In summary, this trial demonstrated clinically meaningful and statistically significant values, and constitutes the first validation of anti-angiogenic therapy in the clinic.

Small molecule VEGFR inhibitors

Dieter Marme (University of Freiburg (Tumor Biology Center), Germany) gave an update report on the development of small-molecule VEGF receptor (VEGFR) inhibitors. Currently, at least ten small-molecule inhibitors of VEGFRs are being evaluated in clinical trials, and others have progressed to preclinical development. Vatalanib (Novartis AG/Schering AG; Figure 1) is currently in phase III trials for colorectal cancer; in one trial ~ 43% of patients demonstrated a partial response, 38% demonstrated stable disease, and the remainder had progressive disease after administration of this drug. Dr Marme also reported that the responder group had lower levels of circulating VEGF, providing a surrogate marker for responsiveness.

Figure 1. The structure of vatalanib.



Therapy for ocular neovascular disease

Retinal vasculopathy of a large range of etiologies has been a disease-target for anti-angiogenic therapy. Eyetech Pharmaceuticals Inc has focused on the development of anti-angiogenic therapy for ocular neovascular disease. Its main target has been VEGF, termed by David Shima (Eyetech Pharmaceuticals Inc, USA) as "the Swiss-knife of vasoactive factors". Dr Shima presented data that support a critical role for VEGF-164 in the development of retinal vasculopathies. Eyetech has developed the aptamer pegaptanib (Macugen, Eyetech Pharmaceuticals Inc/Pfizer Inc) to specifically inactivate the VEGF-164 isoform. Aptamers act similarly to antibodies, bind to the target with high affinity, exhibit remarkable specificity and have low to no immunogenicity. Dr Shima reported results from phase II/III trials that included 1186 patients at 117 centers worldwide. The trials included four treatment arms, placebo or pegaptanib (0.3, 1 or 3 mg) by intravitreal injection, and demonstrated a 27% response rate. Dr Shima also mentioned that trials in patients with diabetic macular edema are ongoing.

Avoiding anti-angiogenic drug resistance or escape

A major concern as the field advances into clinical trials is the possibility of angiogenic drug resistance or escape. Researchers observed this in some patients during the clinical trials of bevacizumab. The remarkable success of

phase III trials in colon cancer contrasts sharply with the outcome of the breast cancer trials. What is the basis for angiogenic drug escape or resistance? Are there alternative molecular mechanisms that can support angiogenesis once VEGF is suppressed? Dr Ferrara addressed these questions in a plenary presentation. Two central highlights of his talk were the contribution of stroma to angiogenesis and the discovery of tissue-specific angiogenesis mediators. On the contribution of stroma, two tumors that contrast significantly in the relative levels of connective tissue were discussed: rhabdomyosarcoma (low) and lung carcinoma (high). Complete inhibition of angiogenesis required the suppression of both tumor- and stroma-derived VEGF. Dr Ferrara also discussed his recent findings on the characterization of a steroid-specific endothelial growth factor. A clear possibility for escape from targeted VEGF therapy is the presence of additional, tissue-specific, growth factors that could compensate for the lack of VEGF. Further research focused on the mechanisms that regulate angiogenesis in a tissue- and tumor-specific manner will be critical to understanding mechanisms of resistance.

Other speakers also addressed the basis of vessel diversity. In particular, the contribution of specific VEGF isoforms to vascular patterning was discussed by Christer Betsholtz (University of Goteborg, Sweden). Using knockin transgenic animals, Dr Betsholtz and colleagues evaluated the effects of splice-specific isoforms in the vascularization of the retina. The central conclusion from these studies is that the spatial distribution of VEGF promotes clues for patterning and branching. In the absence of extracellular matrix anchorage, as is the case with VEGF-121, branching is significantly reduced and most of the vessels terminate abruptly in saccular structures. This is particularly clear in the brain and retina, where extensive branching in one plane is observed. As a result of these experiments Dr Betsholtz was able to further dissect the sprouting vessel. The cell at the leading edge, the 'tip-cell', displays significant filopodia extensions and is highly migratory; however, it does not proliferate. In contrast, cells that constitute the 'trunk' of the sprout do not display filopodia and are highly proliferative. It appears that matrix-bound VEGF is essential for the provision of migratory clues and directionality of the 'tip-cell'. Dr Betsholtz also presented data from mouse models suggesting that certain vasculopathies of the retina can be partially resolved by the use of matrix metalloprotease (MMP) inhibitors. He speculated that MMPs play a role in the release of VEGF from the matrix, and the lack of this binding is responsible, at least in part, for vascular alterations in certain retinopathies. Along these lines, the presentation by Luisa Iruela-Arispe (University of California Los Angeles, USA) demonstrated that MMPs can cleave VEGF-164-releasing bioactive peptides able to phosphorylate VEGFR-2 and induce angiogenesis. The generation of MMP-resistant and cleaved VEGF isoforms had a significant impact on tumor growth and vascular patterning.

Exploring new modalities of therapy

The concept of a metronomic dose of chemotherapeutic drugs, introduced several years ago by Judah Folkman (Childrens Hospital of Boston, USA), was discussed in depth by Robert

Kerbel (Sunnybrook Health Science Center, Canada). Metronomic therapy consists of long-term, frequent or continuous administration of chemotherapeutic drugs at comparatively low doses with no prolonged breaks. The advantages of this modality of therapy are significant and include lower toxicity, an ability to be integrated chronically with targeted cytostatic drugs, and that treatments are less vulnerable to acquired drug resistance. The main disadvantage is its empirical nature. Dr Kerbel presented a wealth of experimental data using tumor xenografts in nude mice that demonstrated that the combined use of several chemotherapeutic drugs with anti-angiogenic agents is highly effective in the suppression of tumor growth. In particular, the utilization of vinblastine and VEGFR-2 antibodies demonstrated sustained tumor regression without overt toxicity. Dr Kerbel also demonstrated that circulating endothelial cell progenitors can be effective surrogate biomarkers for the evaluation of anti-angiogenic drugs.

As vessels are suppressed by anti-angiogenic therapy, the vasculature becomes 'normalized'. This terminology was used by Rakesh Jain (Massachusetts General Hospital, USA) to describe the process by which the highly erratic tumor vasculature is pruned by anti-angiogenesis inhibitors, resulting in a more 'normal' vascular supply. The resulting paradox is that anti-angiogenesis treatment reduces tumor growth while it enhances vascular perfusion. Essentially, blocking the VEGF pathway leads to the destruction of the more destabilized vessels, leaving the stable (basement membrane/pericyte covered) vessels intact, which results in the increased perfusion of blood and overall enhancement in the oxygenation of the tumor. Dr Jain's results solve the apparent paradox by suggesting that the process of normalization also improves the perfusion of chemotactic drugs, resulting in improved distribution and efficacy. He also presented an evaluation of tumor size and tumor vasculature in six patients before and after bevacizumab treatment. Treatment with bevacizumab resulted in a significant reduction of tumor burden in association with reduced vascular supply.

Novel targets

ILK blockade

Shoukat Dedhar (British Columbia Cancer Agency, Canada) presented exciting data on integrin-linked kinase (ILK), a molecule that appears to modulate both VEGF and integrin signaling. Considering the interest surrounding VEGF regulation and the promise of suppressing some integrin pathways, this might be an important target that could potentially combine the effects of both molecules. Suppression of ILK function by homologous recombination has demonstrated that this kinase is essential for embryonic development. Endothelial-specific deletion of ILK using Tie2-Cre mice leads to embryonic lethality between days 9.5 and 10.5 due to vascular defects. Details of the effect on vascular morphogenesis and remodeling, however, were not forthcoming. More pertinent to therapeutic applications, assessment of ILK suppression in tumor xenografts demonstrated a remarkable effect of ILK blockade in the suppression of tumor growth. Experiments were performed using the ILK inhibitor KP-074728 (Kinetek Pharmaceuticals Inc) at a dose of 50 mg/kg in nude mice with tumor xenografts.

HIF-1 α

An elegant presentation dissecting the mechanisms of hypoxia inducible factor (HIF)-1 α hydroxylation was provided by Jacques Pouyssegur (Centre National de la Recherche Scientifique (CNRS), France). HIF-1 α is a critical transcriptional regulator of VEGF; however, it is rapidly degraded by the proteasome system unless hydroxylated. Thus, stabilization of HIF-1 α requires hydroxylation. Three enzymes are responsible for the hydroxylation of HIF-1 α , and their contribution depends on the length of hypoxic stress. These enzymes might constitute an attractive target for anti-angiogenesis, provided the drug is directed toward the tumor cells specifically.

David Cheresch (Scripps Research Institute, USA) discussed the contribution of Src in the permeability responses induced by VEGF. His findings demonstrated that Src inhibition reduces vascular permeability and brain damage following stroke events. Indeed, Dr Cheresch demonstrated that Src $-/-$ mice are protected against stroke. Therefore Src might constitute an important target to suppress permeability responses mediated by VEGF.

Exploiting VEGF as a pro-angiogenic mediator

Eli Keshet (Hebrew University of Jerusalem, Israel) made an eloquent presentation that addressed the therapeutic use of VEGF as a pro-angiogenic mediator. Using double transgenic animals, Dr Keshet has been able to target VEGF expression to specific tissues in an inducible manner. His studies addressed several key questions: how long should VEGF be 'turned on' for the generation of mature/functional vessels, and will neovessels regress after removal of the growth factor? Dr Keshet's experiments targeted the heart and liver and demonstrated that for the generation of neovessels in adult tissues, exposure to VEGF must be limited, as ongoing exposure to VEGF culminates in the formation of hemangioma-like vessels and can lead to the arterIALIZATION of veins. Determination of the time of exposure is also critical to the stability of the neovessels, as

the induced network can regress if VEGF is prematurely withdrawn. Dr Keshet emphasized that separation of the angiogenic and permeability activities of VEGF is essential for the implementation of pro-angiogenic therapies using this growth factor. Dr Keshet also discussed the recruitment of circulating progenitors induced by turning on VEGF. Massive infiltration of bone marrow-derived CD45+ cells were noted in the tissues that overexpressed the growth factor. Prolonged expression of VEGF resulted in bone marrow depletion and extramedullary (liver) hematopoiesis.

Summary

Regulation of vascular growth has recently become an exciting target of cancer research. The possibility of the pharmacological suppression of vascular supply has been validated time and again in several animal models and more recently in clinical trials. Nonetheless, as the field advances new challenges emerge. Would all anti-angiogenic drugs work equally well in all tumors? What is the possible basis for escape from particular anti-angiogenic inhibitors? How might efficacy and delivery be improved? What are the best combinations of inhibitors and chemotherapeutic drugs? These questions can only be answered by a parallel progression in understanding the basic mechanisms that regulate angiogenesis. Increased molecular sophistication in our understanding of blood vessel formation will provide answers to these questions and open new avenues for the development of additional therapeutic approaches. Bevacizumab has been extremely effective in phase III trials with colon cancer. This success renewed confidence in anti-angiogenic therapy for cancer and has supported an increased interest in biotechnology. Cancer, myocardial ischemia, retinal disorders and arthritis have been the main targets for drugs with vascular modulatory effects. However, these are only a few of the pathologies that could benefit from angiogenesis research and the development of drugs to either accelerate or suppress this process. Clearly this is an area of biology that should be monitored for rapid development in both basic science and medicinal chemistry.