Introduction

In the last 9 years, antiangiogenic therapy has become part of standard antitumor treatment. However, the clinical efficacy of such therapies is limited, and it appears that the full therapeutic potential of antiangiogenic intervention has not been fully exploited. Moreover, the mechanistic goals of antiangiogenic intervention are presently less clear than ever. While concepts developed by Folkman in the 1970s suggested that antiangiogenic therapy was supposed to starve tumors to death by driving the tumor-associated vasculature into regression (1), we find that clinically established anti-VEGF/VEGF receptor therapies primarily promote stable disease by preferentially targeting the most immature blood vessels leaving behind normalized and resistant blood vessels (2). The enigmatic question around the importance of vascular regression, tumor vessel normalization, or a spatiotemporal combination of both mechanisms for the efficacy to antiangiogenic intervention has increased to the forefront of basic and applied vascular biology research to guide future pharmaceutical development. One of the most challenging questions at the moment is: What drug combinations improve the clinical efficacy of targeted VEGF pathway therapies without causing toxicity by damaging the mature, resting organ vasculature outside tumors?

Recent Advances in the Mechanistic Understanding of Angiopoietin-2 Function

The vascular receptor tyrosine kinase Tie2 is physiologically activated by its agonistic ligand Ang1. Ang1- and Tie2-deficient mice die in midgestation around embryonic day 10.5 as a consequence of perturbed vessel remodeling and maturation (3). The phenotypic similarity between Ang1 ligand and Tie2 receptor–deficient mice has supported the concept that Ang1 is the single, nonredundant agonistic ligand of Tie2 (4, 5). Using similar logic, the antagonistic mode of action of the second angiopoietin ligand, angiopoietin-2 (Ang2), was inferred from genetic manipulation experiments in mice: Ang2-overexpressing transgenic mice essentially phenocopy the midgestational embryonic lethal phenotype of Ang1-deficient mice (6). In other words, too much Ang2 resembles too little Ang1. In addition, correlative expression of Ang2 at times of vessel regression in ovaries, tumor vascular cooption, and hyaloid vessel regression supported the concept that the dominant biologic role of Ang2 is destabilization of established blood vessels through the interruption of Tie2 signaling, as a prerequisite to sprouting angiogenesis in the presence of proangiogenic factors.
Deletion of Tie1 in mice results in defects of vascular integrity, localized hemorrhaging, and cardiac underdevelopment, indicating its essential role in vascular maintenance (22). Tie1 is highly expressed by arteries and their associated capillaries at branching points and is induced by disturbed flow, suggesting dynamic physiologic regulation of Tie1 (23). Multiple studies have addressed potential cross-talk between Tie1 and Tie2, as well as feedback regulatory loops that respond differently to Ang1 and Ang2 (24, 25). These data are still emerging but a take-home hypothesis generated by Seegar and colleagues suggests that in the presence of Tie1, Ang2 is unable to activate Tie2; however, loss of Tie1 reveals the agonist capabilities of Ang2 in vitro (26). These tantalizing data await a more physiologic context and confirmation in vivo.

Ang2 has recently been shown to exert proangiogenic functions in a Tie2-independent manner. Angiogenic endothelial tip cells downregulate the Tie2 receptor and Ang2 signals in these cells through activated integrins to promote focal adhesion kinase (FAK) signaling and, subsequently, angiogenic sprouting (27). While not acting through an agonism of Tie2, this is nevertheless an unexpected proangiogenic function of Ang2 where neutralization of the ligand could be beneficial in a cancer context. It is notable that in terms of Ang2 functions, both its role in destabilizing a mature vasculature to facilitate cooption and neoangiogenesis and its role in promoting integrin stimulation of sprout- ing tip cells suggest that the consequences of Ang2 blockade will likely be contextual with different net outcomes on sprouting Tie2-negative tip cells and more mature Tie2-expressing stalk and phalanx endothelial cells (see Fig. 1). This is consistent with published reports using neutralizing molecules to Ang2 where tumor growth and retinal sprouting are blocked (e.g., ref. 28). These studies use antibodies that were developed to block Tie2 interactions and often to block the in vitro activation of Tie2 that can be observed in the absence of Ang1, suggesting that the antibodies or peptides block Ang2–Tie2-interacting domain. It is unclear whether the observed Ang2 interactions with integrins use the same binding domains and whether this function is sensitive to the currently available blocking antibodies and peptides to Ang2 in therapeutic development described below.

In a recent issue of Cancer Research, Daly and colleagues reveal insights into the complexity of Ang/Tie signaling that provides a model for the contextuality of Ang2-antagonistic and agonistic functions of Ang2 on Tie2 (Fig. 1). The authors show that a selective antibody to Ang2 (REGN910) can decrease tumor growth in a manner that is reversed by high-dose administration of an engineered form of Ang1 called Ang1-F1-Fc-F1 (which has an IC50 of 9.7 nmol/L to Tie2 and is a forced tetramer), hinting that Tie2 signaling was attenuated (29, 30). This Ang1 construct has previously been shown to maximally activate Tie2 in vitro and in vivo and to block mustard oil–induced vascular leak. While this report falls short of directly showing the impact of REGN910 on activation of Tie2 by Ang2 in vivo, the tumor growth and resulting gene expression data presented, along with the above described cellular contexts in which Ang2 can have proangiogenic and
Angiopoietin-2 as Antiangiogenic Target

Figure 1. Endothelial cell phenotypes during tumor angiogenesis. A, endothelial cells during angiogenesis maintain distinct functions and acquire specific phenotypes during the individual steps of the angiogenic cascade. Sprouting tip cells (1) have an invasive filopodia-rich promigratory phenotype. They sense the gradient of angiogenic cytokines and migrate towards the angiogenic stimuli. Tip cells are followed by stalk cells (2), which proliferate and remodel to establish lumen-forming microvessels. Tip cell– and stalk cell–derived PDGFβ is the best characterized pericyte-recruiting cytokine during angiogenesis. Pericyte recruitment leads to microvessel maturation. Loosely attached pericytes cover remodeling phalanx cells (3). Fully mature phalanx endothelial cells (4) are in intimate contact with pericytes, which control the quiescent endothelial cell phenotype. B, application of the tip cell/stalk cell/phalanx cell model of angiogenesis applied to the partial Ang2 agonist concept for cancer: The mature organ vasculature outside tumors (4) expresses Tie2, and the cells are surrounded by Ang1-producing pericytes. There is low-level Ang1 production, which is stored in quiescent phalanx endothelial cells, that can be induced and secreted upon activation. Release of Ang2 would likely result in a quenching of Ang1/Tie2 signal transduction indicative of an antagonistic mode of action. Conversely, the most immature tip cell vasculature (1) downregulates Tie2 and upregulates Ang2 creating a milieu in which Ang2 can signal agonistically through integrins. The exact positional information on Ang ligands and Tie receptors in stalk cells and in remodeling phalanx cells awaits further analysis, but it is most likely that Tie2- and Ang2-expressing endothelial cells in a low-Ang1 environment could be found in this zone (2,3) where the partial agonist Ang2 results in a net activation for Tie2. The function and the positional expression of the Ang/Tie2 response, including the role of Tie1 in this context, similarly await further clarification.

possibly even Tie2-agonistic activity, provide a compelling case to revisit the simplistic view of the Yin-Yang of Ang1 and Ang2.

The data presented in the article by Daly and colleagues are compatible with a partial agonist model of Ang2 function. In line with classical pharmacologic concepts, the partial agonist competes with the strong agonist. In the presence of the strong agonist (in this case, Ang1), the partial agonist Ang2 quenches the signal induced by the strong agonist, thereby exerting an inhibitory activity. In the absence of the strong agonist, the weak agonist may function as receptor-stimulating ligand. This model of Ang2 as a partial agonist can perhaps provide a unifying hypothesis to explain some of the contextuality of Ang/Tie biology. For example, the embryonic lethal phenotype of Ang1-deficient mice may be explained by the fact that Ang2 is only later expressed in endothelial cells during embryonic development. Conversely, the puzzling observation that Ang1 deletion in adult mice did not result in overt pathology unless the mice were pathologically challenged (31) may well be attributed by a compensatory agonistic function elicited by the partial antagonist Ang2, which would be a testable hypothesis. Clearly, the model proposed by Daly and colleagues provokes a long list of experiments on the contextuality of Ang/Tie function, which emerges at a time when better reagents for the tracing of receptor and ligand expression and for the conditional mutagenesis of the ligands and receptors becomes more readily available. An accelerated pace in the elucidation of the molecular mechanisms of Ang/Tie signaling in the tumor context holds great promise to enable the rational translation of Ang/Tie pathway therapeutics into clinical application.

Current Therapeutics Targeting the Angiopoietin and Tie2 Pathway

There are no selective Tie2 kinase inhibitors in clinical development; however, several approved agents show cellular activity against this pathway. Regorafenib is one of several small-molecule VEGFR2 inhibitors that also exhibit some activity against Tie2. Regorafenib has a 3 to 10 nmol/L cellular activity against VEGFR2, Ret, Kit, and Raf and modest (30–150 nmol/L) cellular activity against fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), and Tie2. Phase III trials showed a significant increase in progression-free survival (PFS) and overall survival in second-line metastatic colorectal cancer (CRC) leading to the accelerated approval of the drug (32). Cabozanib, a related VEGFR TKIs that is also active against Ret, Tie2, and other kinases, is now approved in medullary thyroid cancer (33). Similarly, MGCD-265 is a Ron, Tie2, cMET, and VEGFR1/2/3 inhibitor that is being currently tested in phase II trials in patients with a variety of advanced metastatic malignancies (34). In addition, there are several small-molecule inhibitors (ACTB-1003, CEP-11981, ARRY-614) in phase I trials. As it is unlikely that any of these molecules has dominant impact via Tie2 signaling, the remainder of this discussion will focus on the variety of more selective biologics currently under development.

There are 2 types of biologics targeting the angiopoietins whose references can be found in Table 1. The first and most advanced are peptide based, using peptides with high affinity and differing selectivity to Ang1 and Ang2. These peptides are either linked to an Fc domain or to an irrelevant antibody. Trebananib (AMG-386) and PF04856884 (CVX-060) are the 2 peptide-based traps that are most advanced, in phase III and 2 trials, respectively.
Table 1. Therapeutics targeting Ang-Tie axis

<table>
<thead>
<tr>
<th>Company</th>
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<th>Highest phase of development</th>
<th>Clinical data</th>
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<tr>
<td><strong>Small-molecule inhibitors</strong></td>
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<tr>
<td>Regorafenib</td>
<td>FGFR, Kit, Tie2, VEGFR1,2,3, RET, BRAF, CRAF, PDGFR and caspase-3/9 stimulator</td>
<td>Phase III</td>
<td>Positive phase III trials (significant increase in PFS) in second-line metastatic CRC and third-line gastrointestinal stromal tumors (46).</td>
<td>Showed inhibition of tumor growth in several tumor xenograft models including pancreatic, lung, colon carcinoma and regression in breast carcinoma model (47). In addition showed a significant increase in survival with fewer metastasis in syngeneic MC38 CRC liver metastasis model (48).</td>
</tr>
<tr>
<td>Cabozantinib (XL-184/BMS-907351)</td>
<td>MET, VEGFR2, RET, Kit, FLT3, Tie2</td>
<td>Phase III</td>
<td>Positive phase III trials in medullary thyroid cancer and ongoing phase III trials in metastatic castration-resistant prostate cancer.</td>
<td>Reduced metastasis in RIP-TAG2 model of pancreatic neuroendocrine carcinoma compared with VEGF inhibitor alone (49).</td>
</tr>
<tr>
<td>MGCD-265</td>
<td>RON, Tie2, VEGFR1, 2, 3, cMET</td>
<td>Phase II</td>
<td>A parallel-arm, open-label trial in advanced metastatic malignancies and NSCLC began for assessing antitumor activity, PFS, and safety (50).</td>
<td>Showed tumor regression in MKN45 gastric cancer xenograft model in combination either with erlotinib or docetaxel. Showed antitumor activity and tumor growth regression in MET-driven human xenograft cells (gastric carcinoma) and non–MET-driven tumor cells (NSCLC and prostate) in combination with paclitaxel and docetaxel (34).</td>
</tr>
<tr>
<td>Foretinib</td>
<td>MET,VEGFR1, 2, Tie2, RON, AXL, FLT3 and PDGFR-beta</td>
<td>Phase 2</td>
<td>A phase1/2 trial in HCC and phase 2 trials in recurrent or metastatic squamous cell head and neck cancer, metastatic gastric cancer and hereditary or sporadic papillary RCC (NCT00725764, NCT00743067, NCT00920192).</td>
<td>Dose-dependently inhibited tumor growth in several models including glioblastoma, breast, CRC, and NSCLC (51).</td>
</tr>
<tr>
<td>ACTB-1003</td>
<td>VEGFR2, Tie2, RSK, FGFR1 and S6K</td>
<td>Phase I</td>
<td>None.</td>
<td>Showed tumor growth inhibition in cell lines with FGFR genetic alterations—OPM2 human multiple myeloma and the murine leukemia Ba/F3-TEL-FGFR1 and in H460 (52).</td>
</tr>
<tr>
<td>CEP-11981</td>
<td>Tie2, VEGFR1, 2,3, FGFR1</td>
<td>Phase I</td>
<td>Linear, dose-related increases in plasma pharmacokinetic exposure (mean $C_{\text{max}}$ and AUC) with oral administration. Exposure-related increases in VEGFR2 inhibition ex vivo were shown in a human plasma-based cellular Bioassay (NCT00875264).</td>
<td>Dose-dependent tumor growth inhibition in U251 MG human glioblastoma xenograft and A375 human melanoma xenograft model (53).</td>
</tr>
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Table 1. Therapeutics targeting Ang-Tie axis (Cont’d)

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<tr>
<td>ARRAY-614</td>
<td>Array BioPharma, Inc.</td>
<td>p38, Tie2</td>
<td>Drug was well tolerated and the MTD was not reached with QD dosing. Recently tested a new formulation with enhanced drug exposure and lower variability for future trials (54).</td>
<td>Antitumor activity shown in multiple myeloma in combination with lenalidomide. Complete regressions in BCR-Abl-driven model of chronic myeloid leukemia (55).</td>
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<tr>
<td><strong>Large-molecule inhibitors</strong></td>
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<tr>
<td>Trebananib-peptide: Fc fusion-peptibody, AMG-386</td>
<td>Amgen</td>
<td>Ang1 and Ang2</td>
<td>No improvement in PFS in combination with sorafenib in metastatic RCC (phase II; ref. 36). Evidence of antitumor activity and a dose-response effect in combination with paclitaxel in advanced recurrent epithelial ovarian or primary peritoneal cancer (37). No apparent prolongation of PFS in combination with paclitaxel in HER2-negative, metastatic, or locally recurrent breast cancer (56).</td>
<td>Significantly inhibited tumor growth of several tumor types, including Colo205 (CRC) and A431 (epidermoid carcinoma). A dose-dependent antiangiogenic effect in VEGF-mediated rat corneal angiogenesis assay. Had greater tumor growth suppression than inhibiting Ang1 or Ang2 alone (57).</td>
</tr>
<tr>
<td>CVX-060/ PF04856884, recombinant humanized CovX body</td>
<td>CovX Pharmaceuticals (Pfizer)</td>
<td>Ang2</td>
<td>A randomized, crossover, open-label phase II trial in metastatic RCC in combination with axitinib was initiated (NCT01441414).</td>
<td>As efficacious as bevacizumab in a colon cancer xenograft model and found to improve the response to irinotecan or docetaxel when administered together (35).</td>
</tr>
<tr>
<td>AMG-780, fully human anti-Ang1/2 mAb</td>
<td>Amgen</td>
<td>Inhibits Tie2-Ang1/2 interaction</td>
<td>Dose-escalation and expansion in patients with advanced solid malignancies (NCT01137552).</td>
<td>None.</td>
</tr>
<tr>
<td>MEDI-3617, fully human anti-Ang 2 mAb(3.19.3)</td>
<td>AstraZeneca</td>
<td>Ang2 (Ang1 weakly)</td>
<td>Dose-escalation in patients with advanced solid malignancies. Modest toxicity was observed. No objective response. Five of 17 evaluated patients had stable disease at 12 wks (58).</td>
<td>Inhibited tumor growth in mice with human pancreatic and hepatocellular xenografts. Efficacy enhanced in combination with sorafenib and bevacizumab. Efficacy also enhanced in human CRC models in combination with vandetanib, cediranib, or DC101 (59).</td>
</tr>
<tr>
<td>REGN-910, fully human anti-Ang2 mAb</td>
<td>Sanofi/ Regeneron</td>
<td>Ang2</td>
<td>Dose-escalation and expansion study in patients with advanced solid malignancies (60).</td>
<td>Significantly inhibited growth of several tumors types including PC3 (prostate), Colo205 (CRC), and A431 (epidermoid carcinoma). Reduced Colo205 tumor vascularity and tumor perfusion more than single agents, as per histologic and microultrasound analyses (61). Potentiates the effects of VEGF-Trap (Aflibercept; ref. 62).</td>
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(Continued on the following page)
Trebananib is a peptide–Fc fusion protein (peptibody) that blocks the interaction of both Ang1 and Ang2 to Tie2 with an affinity of 0.67 nmol/L by BiAcore (35). Several phase II trials were conducted with trebananib in which some hints of clinical efficacy and experience with pharmacokinetics (PK)/pharmacodynamics (PD) has led to further development in phase III, particularly in ovarian cancer. Phase II data showed an improved response rate but no difference in PFS when trebananib was combined with sorafenib in patients with metastatic clear cell renal carcinoma (RCC; ref. 36). However, an encouraging dose–response effect was observed when trebananib was used in combination with paclitaxel in patients with advanced recurrent epithelial ovarian or primary peritoneal cancer (37). PK/PD analysis of the phase II data suggest that maximum benefit in PFS may not have been reached with a trebananib dose of 10 mg/kg once every week and predict that a dose of 15 mg/kg once every week may provide greater exposure and may yield further improvements in PFS (38). The 3 phase III trials now ongoing at 15 mg/kg once-a-week dose of trebananib combine either paclitaxel (NCT01204749/TRINOVA-1), pegylated liposomal doxorubicin (NCT01281254/TRINOVA-2), or a combination with paclitaxel and carboplatin (NCT01493505/TRINOVA-3). TRINOVA-1 and -2 are in recurrent platinum-sensitive or -resistant epithelial ovarian, primary peritoneal, or fallopian tube cancers, whereas TRINOVA-3 is in first-line FIGO Stage III-IV epithelial ovarian, primary peritoneal, or fallopian tube cancers (39).

PF04856884 (CVX-060) is a CovX-body specific to Ang2. CovX-bodies are an antibody platform that are generated by linking an irrelevant (aldolase) IgG to a target-binding peptide. CVX-060 has a binding affinity of 4.9 nmol/L to Ang2, similar to published affinity for Ang2 to Tie2. The peptide is linked using a branched azetidinone (AZD). The antibody scaffold imparts IgG-like half-life, and distribution and the peptide pharmacophores are responsible for functional activities (6, 40). CVX-060 inhibited tumor growth, tumor microvessel density, and intratumor proangiogenic Tie2/CD11b-positive cells (TEMs) in Colo205 xenograft tumors. Even greater efficacy (>80%) was observed when combined with sunitinib, sorafenib, bevacizumab, irinotecan, or docetaxel in this model (35). Significant mean decreases in tumor blood flow assessed by $K_{trans}$ (DCE-MRI) and increase in serum Ang2 levels were observed in a phase I study of CVX-060. The maximum tolerated dose (MTD) was not defined and the most common adverse event was fatigue. PK/PD analysis has recommended 15 mg/kg once weekly dosing (41). It is unclear whether increased serum Ang2 levels in this study are due to peptibody binding and accumulation of stabilized complexes in the circulation as opposed to a pharmacodynamics induction of de novo Ang2

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<tbody>
<tr>
<td>TAvi6, a CrossMab tetravalent IgG-like bispecific antibody</td>
<td>Roche Ang2 and VEGF-A</td>
<td>Discovery</td>
<td>None.</td>
<td>Effectively reduced angiogenesis, tumor growth, and metastasis in several subcutaneous and orthotopic in vivo models that are resistant to anti-VEGF treatment. Ang2-selective approach was found to have safety-related advantages over simultaneous Ang1 and Ang2 inhibition (63).</td>
</tr>
<tr>
<td>AT-006, fully human mab</td>
<td>Roche/ Affitech Ang2</td>
<td>Discovery</td>
<td>None.</td>
<td>Inhibited tumor growth and neoangiogenesis (64).</td>
</tr>
<tr>
<td>Anti-Her2/anti-Ang2 mab, multivalent mab</td>
<td>Zynegia Her2 and Ang2</td>
<td>Discovery</td>
<td>None.</td>
<td>Remained intact and functional in mouse serum and can bind to Her2 and Ang2 simultaneously (65).</td>
</tr>
<tr>
<td>A-11, a peptide inhibitor of Ang2 and Tie2 interaction</td>
<td>Compugen Ang2</td>
<td>Discovery</td>
<td>None.</td>
<td>Inhibitedretinal neovascularization in a rat model of oxygen-induced retinopathy. Showed synergy with anti-VEGF antibody (66).</td>
</tr>
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Abbreviations: NSCLC, non–small cell lung cancer; qd, once daily.

expression. A phase II study is ongoing in combination with axitinib in patients with previously treated metastatic RCC (NCT01441414; ref. 39). In general, peptides tend to have lower affinities for Ang2 binding than antibodies, and understanding how competitive they are as neutralizing agents clinically awaits further development of the antibodies described below.

The second type of biologic therapeutics are antibodies. Two selective anti-Ang2 antibodies are in development, MEDI-3617 (AstraZeneca) and REGN-910 (Sanofi). Both MEDI-3617 and REGN-910 have ongoing phase I dose-escalation studies as single agents or in combination with other chemotherapy in patients with advanced solid tumors (NCT01248949 and NCT01271972). MEDI-3617 reported drug-related adverse events (% of patients) including fatigue (24%), diarrhea (19%), nausea (19%), dysgeusia (14%), and headache (14%), all of which were ≤ grade II (42). AMG-780, an antibody that binds both Ang1 and Ang2, is also in phase I development (NCT01137552) (39). In addition, Roche has a preclinical stage CrossMab, which is a bispecific antibody that binds and neutralizes both Ang2 and VEGF-A. This bifunctional approach is notable, as several preclinical studies have shown an advantage to dual inhibition of both VEGF/VEGFR and Ang/Tie2 pathways, including the study by Daly and colleagues, in a recent issue of Cancer Research (29, 42).

Outstanding Questions and Future Directions for Rational Intervention

The prominent upregulation of Ang2 in the vasculature of most solid tumors makes Ang2 an attractive candidate for therapeutic intervention. Preclinical and clinical studies suggest that Ang2 may augment the activity of VEGF-targeted therapies without clear evidence of enhanced toxicity. However, the pleiotropic activities of Ang2 in blood, lymphatic, and myeloid biology, as well as complexities in receptor use and contextual presentation demand further investigation to clarify the critical aspects of Ang2 that are drivers in cancer progression. Regardless of which effects turn out to be dominant in cancer, current evidence supports the therapeutic rationale to antagonize Ang2.

In tumor angiogenesis, Ang2 appears to function as a potent Tie2 agonist based on the ability of Ang1 to counter the efficacy of anti-Ang2 antibodies. However, modulation of Tie2 activation in tumor vessels remains to be explored (29). Ectopic administration of Ang1 can substitute for Ang2 function, raising the question of the difference between autocrine Ang2 (released by endothelial cells subjected to stress) or paracrine Ang2. This may be particularly relevant in some tumor settings where Ang2 is produced by tumor cells. Ang2 has also been associated with tumor metastases. High Ang2 expression in primary human breast cancer specimens correlates with an increased metastatic phenotype (44). Furthermore, overexpression of Ang2 in MCF-7 promoted their metastasis to lymph nodes and lungs, an effect postulated to result from direct interaction between Ang2 and beta1 integrins on tumor cells (much the way Ang2 was reported to facilitate sprouting of Tie2-negative tip cells; refs. 11, 27). The delineation of autocrine versus paracrine presentation of Ang2 still needs to be carefully investigated.

The partial agonist concept developed by Daly and colleagues raises important questions as to when and where angiopoietin ligands and Tie2 receptor are expressed in different tumor settings (Fig. 1; ref. 1). Endothelial cells in mature tumor-associated vessels express Tie2 in an Ang1-rich/Ang2-poor microenvironment. When this balance is disturbed by increased expression of Ang2 (a weak agonist), the net result is a paradoxical decrease in Tie2 phosphorylation. Conversely, endothelial cells in the most immature tumor vasculature downregulate Tie2 expression, while at the same time upregulating Ang2 and favoring proangiogenic Ang2 function through integrin signaling. The contextual presentation of Ang1 in tumors is not well-defined, but an intermediate zone of Tie2- and Ang2-expressing endothelial cells in an Ang1-low microenvironment could likely be present in the actively proliferating stalk cell zone (Fig. 1).

Extrapolating from rapidly growing mouse tumors with a very immature vasculature, it is widely believed that the tumor vasculature is low in Ang1-producing pericytes. However, this is most likely an overstatement as microvessels in human tumors are often well-invested by pericytes. These pericytes may not be as tightly associated with endothelial cells as in resting blood vessels but are widely detected in human tumors (45). In fact, there are distinct differences in the pericyte coverage of different human tumor blood vessels. It is tempting, therefore, to speculate that pericyte-mediated differences in tumor blood vessel maturation could account for differences in the efficacy of immature blood vessels targeted by anti-VEGF/VEGFR therapies. These concepts strongly support a rationale for inducing vascular maturation as part of the treatment of tumors.

The development of Ang/Tie pathway drugs and the deciphering of the intricate details of Ang/Tie signaling occur at a time when the goals of antiangiogenic intervention are under serious consideration. Understanding the cross-talk between vessel maturation and remodeling (i.e., Ang/Tie) and primarily sprouting angiogenesis-inducing pathways (i.e., VEGF) holds great promise to rationally develop safe and improved antiangiogenic cancer therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: D. Gerald, S. Chintharlapalli, H.G. Augustin, L.E. Benjamin

Writing, review, and/or revision of the manuscript: D. Gerald, S. Chintharlapalli, H.G. Augustin, L.E. Benjamin

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Gerald, L.E. Benjamin

Acknowledgments

The authors thank Greg Flowman for edits and helpful suggestions.

Received December 27, 2012; accepted January 9, 2013; published OnlineFirst March 6, 2013.
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Angiopoietin-2: An Attractive Target for Improved Antiangiogenic Tumor Therapy


Cancer Res. Published OnlineFirst March 6, 2013.

Updated version

Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-12-4697

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