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Angiopoietin2 and Tie2: Tied to Lymphangiogenesis and Lung Metastasis. New Perspectives in Antimetastatic Antiangiogenic Therapy

Adriana Albini, Douglas M. Noonan

Correspondence to: Adriana Albini, PhD, Oncology Research Laboratory, IRCCS MultiMedica, Via Fantoli 16/15, Milan 20138, Italy.

The development of new blood and lymphatic vessels from preexisting ones is recognized as essential for tumor progression and metastasis; therefore, angiogenesis inhibition is finding increasing application in the clinic. The most studied signaling pathway for angiogenesis, and the one to which most of the efforts of the pharmaceutical industry have been devoted, is that of the vascular endothelial growth factor (VEGF) and its receptors, mainly VEGFR2. However, angiopoietins 1 and 2 (Ang1/2) and their receptor Tie2 represent another major tyrosine kinase-ligand system that is fundamental for the regulation of angiogenesis (1,2).

Ang2 is part of the Tie/angiopoeitin signaling pathway involved in vascular growth and maturation. In the presence of the Tie2 agonist Ang1, Ang2 may act as an antagonist, blocking Tie2 signaling, although the actions of the angiopioetins are largely context dependent (1,2). Activation of Ang1/Tie2 signaling ensures structural integrity of mature vessels and protects the endothelium from activation by cytokines, whereas Ang2/Tie2 signaling promotes vascular destabilization and enables VEGF-induced angiogenesis (1). Because Ang2 mediates endothelial cell (EC) responsiveness to tumor-derived cytokines, its targeting has promising features for antiangiogenic cancer therapy (2). Furthermore, expression of Ang2, like that of VEGF, is induced by hypoxia, a hallmark feature of most cancers.

Both the VEGF and Ang receptor systems are expressed on ECs. Ang2 is primarily produced by ECs and is strongly induced upon EC activation, indicating a regulatory role of the local microenvironment (3). However, VEGFs and Ang2 are also expressed by a variety of cancer cells (4,5). Tie2 signaling has a key role in lymphangiogenesis (1). Thus, both VEGF and Ang systems hold a pivotal interest for the control of angiogenesis-associated disease, although development of agents targeting the Ang/Tie system lags behind.

Most clinically approved approaches target the main angiogenesis axis, VEGF/VEGFR. In 2009, several investigators brought up a major caveat in angiogenesis inhibition, which hangs like the sword of Damocles over the future of antiangiogenic therapy. The groups of Oriol Casanovas (Catalan Institute of Oncology, L'Hospitalet de Llobregat, Spain) and Robert Kerbel (Sunnybrook Health Sciences Centre, Toronto, Canada) both found that VEGF-targeted angiogenesis inhibition increased metastasis in experimental models (6,7), apparently due to hypoxia-induced overexpression of the *met* axis components (8). Clinicians have not yet reported this effect, perhaps in keeping with the vascular normalization hypothesis of Jain (9). However, nearly all angiogenesis inhibitory therapies are approved for advanced disease. As angiogenesis inhibition moves further up the line in therapeutic strategies, theoretically, the risk increases for clinical observation of enhancement of metastatic dissemination.

In this issue of the Journal, Holopainen et al. (10) report that specific targeting of Ang2 inhibits angiogenesis and metastasis in mouse tumor models (10). Blocking the Ang/Tie2 pathway by bivalent anti-Ang1/Ang2 antibodies was previously shown to curb tumor growth (11). Holopainen et al. (10) show that systemic overexpression of Ang2 promotes metastatic dissemination, whereas specific Ang2 blockade represses it. The authors investigated the antimetastatic effects of the Ang2 blockade and found that it attenuated tumor lymphangiogenesis and reduced tumor cell dissemination into the regional lymph nodes, a major route for metastasis. They also found that metastases are associated with areas of blood vessel disruption, as evidenced by endothelial junctional alterations and detachment from the basement membrane. Importantly, the Ang2 blockade reverted these vascular phenotypes that were associated with metastasis.

These data add to a rapidly expanding series of indications that targeting Ang2 may be a key alternative (or complementary) strategy for counteracting the pro-metastatic activity of certain antiangiogenic approaches (6,7). In an independent study, Mazzieri et al. (12) recently showed that an Ang2 blockade represses tumor angiogenesis and metastasis in mouse models of spontaneous carcinogenesis, including metastatic breast cancer (12). In addition to direct effects on the tumor blood vessels, these authors focused on the role of Ang2 in modulating the activity of proangiogenic Tie2-expressing macrophages (TEMs). There is substantial evidence that TEMs are regulators of tumor angiogenesis in vivo (1) and that these cells respond to Ang2 in vitro (13,14). Although TEMs were recruited into the tumors, inhibition of Ang2 appeared to interrupt a feed-forward loop involving Tie2 upregulation on TEMs that was sufficient to interfere with their ability to promote tumor blood vessels (12). Whereas both studies (10,12) show that Ang2 blockade inhibits dissemination of metastases, Mazzieri et al. (12) also found that when performed after tumor cell dissemination, Ang2 inhibition could hinder further growth of established metastatic nodules. Although Mazzieri et al. (12) did not investigate the mechanisms of metastasis inhibition in their study (12), it is possible that TEMs directly promote the growth of metastasis by inducing

angiogenesis. Further evidence for a role of Ang2 in metastatic dissemination comes from its association with metastasis and chronic inflammation, another hallmark of cancer, in a chemical carcinogenesis model (15).

Our views on metastasis are undergoing rapid evolution, if not a complete revolution (8). While the role of EC disruption in mediating metastatic dissemination seems intuitive, an important role of myeloid cells is being uncovered (16–18). Tumor-infiltrating myeloid cells promote tumor cell intravasation, dissemination, and metastasis (16,17). Furthermore, they are increasingly linked to the formation of the "pre-metastatic niche," a local microenvironment induced by the primary tumor before the arrival of metastatic cells to the distant organ (19). Ang2-mediated EC destabilization and TEM activation may have a key role in the formation of the pre-metastatic niche (20). Future challenges include understanding the role of TEMs in building and modulating the pre-metastatic niche and the role of Ang2 effects on ECs for recruitment and activation of myeloid cells. Together, these novel findings suggest that the production of VEGF and Ang2 by tumors has both local and systemic effects (Figure 1). Local effects of Ang2 include promotion of lymphangiogenesis and lymphatic tumor cell dissemination (10), whereas systemic Ang2 effects may involve the "preparation" of pre-metastatic niches. In addition, Ang2 inhibition appears to prevent myeloid-mediated angiogenesis (12), a main mechanism of resistance (21,22).

Holopainen et al. (10) also show that blocking Ang2 inhibits developmental retinal vascularization. This suggests that Ang2 may be an interesting target in other angiogenesis-associated diseases, including ocular and cardiovascular diseases.

From a clinical point of view, one wonders if Ang2 blockade can alleviate the concerns of hypoxia-induced metastatic dissemination. Given the context-dependent functions of Angs, several points still need to be investigated: 1) Would blocking Ang2 alone be more advantageous than blocking both Ang1 and Ang2, the current main strategy of the few clinical trials? 2) Should we block only Ang2 or both Ang2 and VEGF? 3) Would Tie2 inhibition by small molecule inhibitors be a "risky business" or an ideal strategy? 4) When should anti-Ang2 treatment start to efficiently block metastasis?

Finally, the majority of current antimetastatic drug studies are conducted using cultured tumor cells and preclinical models, measuring the capability to impair growth or invasion in vitro, to reduce tumor growth or prevent metastasis in vivo. However, with standard approaches to clinical trials, although we can study drugs able to reduce tumor size and/or metastatic burden, it is difficult to study drugs that prevent metastasis. With our current clinical trial designs, it is likely that many potentially useful "purely" antimetastatic drugs have been overlooked or discarded. In our view, targeting Ang2/Tie2 deserves additional attention for further therapeutic development in adequate clinical trials.

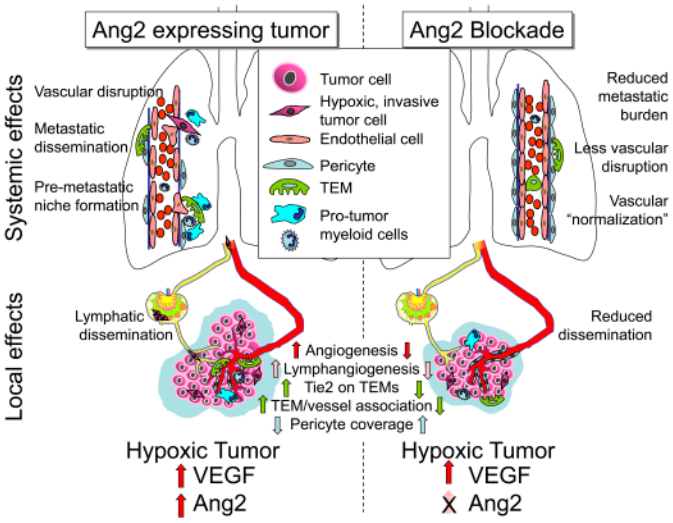

Figure 1. Hypothetical roles of Ang2 in metastasis and the pre-metastatic niche based on recent studies and the effects of Ang2 blockade on dissemination and development of metastasis. Ang2 = angiopoeitin 2; TEM = Tie2-expressing macrophage; Tie2 = tyrosine kinase with immunoglobulin and epidermal growth factor homology domains; VEGF = vascular endothelial growth factor.

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Notes

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Affiliations of authors: Oncology Research Laboratory, IRCCS MultiMedica, Milan, Italy (AA, DMN); MultiMedica, Castellanza (VA), Italy (AA); Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy (DMN).