Beyond Angiogenesis: Exploiting Angiocrine Factors to Restrict Tumor Progression and Metastasis

Mahak Singhal[1,2,3] and Hellmut G. Augustin[1,2,4]

ABSTRACT

Looking beyond tumor angiogenesis, the past decade has witnessed a fundamental change of paradigm with the discovery that the vascular endothelium does not just respond to exogenous cytokines, but exerts active "angiocrine" gatekeeper roles, controlling their microenvironment in an instructive manner. While vascular niches host disseminated cancer cells and promote their stemness, endothelial cell–derived angiocrine signals orchestrate a favorable immune milieu to facilitate metastatic growth. Here, we discuss recent advances in the field of tumor microenvironment research and propose angiocrine signals as promising targets of future mechanism-driven antimetastatic therapies, which may prove useful to synergistically combine with chemotherapy and immunotherapy.

Introduction

The limited diffusion distance of oxygen into tissues (100–150 μm) restricts the growth of tumor cells away from blood vessels. This biophysical phenomenon led Judah Folkman in 1971 to postulate that tumor growth may be angiogenesis-dependent and, conversely, that inhibition of tumor neovascularization could restrain tumor growth to microscopic size in an avascular dormant state. Intense research in the following two decades resulted in the identification of pleiotropic and specific growth factors capable of inducing tumor neoangiogenesis. Notably, VEGF was identified by Napoleone Ferrara in 1989 as the most specific, hierarchically high master inducer of the angiogenic cascade. Blocking reagents to the VEGF–VEGF receptor signaling axis were developed soon thereafter and, in line with Folkman's hypothesis, proved highly potent to suppress the growth of xenografted tumors in mice. Concurrently, bevacizumab, a humanized anti-VEGF antibody, in combination with chemotherapy, prolonged the overall survival of patients with metastatic colorectal carcinoma, paving the way for the FDA approval of the first antiangiogenic therapy. While the most remarkable efficacy of preclinical trials could not similarly translate in man, drugs targeting the VEGF/VEGFR axis have received FDA approval as first-line therapy in combination with chemotherapy for multiple types of cancer, leading on average to an extension of overall survival in the range of 25%.

Antiangiogenic drugs have now been in clinical use in the field of oncology for more than 15 years. Still, the precise mechanism-of-action and their effect on the tumor microenvironment remain elusive to this day. It is poorly understood why antiangiogenic drugs synergize with chemotherapy. Regression of intratumoral blood vessels should, if anything, reduce the delivery of cytotoxic drugs to a tumor. To reconcile for this apparent discrepancy of preclinical concept from clinical reality, Rakesh Jain proposed that antiangiogenic drugs may not necessarily drive all intratumoral blood vessels in regression, but preferentially the highly irregular, tortuous, and poorly functioning immature microvasculature. As a result, the more mature vasculature may persist, resulting in a "normalizing" effect of the remaining, better perfused vasculature. This reduces intratumoral fluid pressure and eventually leads to enhanced drug delivery. While the concepts of vascular normalization are today widely accepted, the actual relative contribution of vessel normalization and vessel regression toward the efficacy of antiangiogenic drugs in human tumors continues to be poorly understood, which is a major bottleneck to rationally advance the clinical efficacy of approved antiangiogenic drugs, for example, by combination with second-generation antiangiogenic drugs.

Beyond acting as responsive cell population executing exogenously stimulated programs, such as angiogenic or inflammatory activation, endothelial cells have in the last decade been recognized as a highly dynamic cell population that serves as a source of instructive signals for neighboring cells. For example, endothelial cells may control embryonic organogenesis even prior to the establishment of a functional circulatory system. Extrapolating these developmental findings into the adult, Shahin Rafii proposed in 2010 that organ-specific endothelial cells may actively shape the local tissue microenvironment by releasing a wide-array of paracrine/juxtacrine-acting growth factors. This novel type of instructive, vascular-controlled cell–cell communication was subsequently referred to as "angiocrine signaling." These newly discovered perfusion-independent molecular functions of the vascular endothelium quickly emerged into the much broader concept of organotypic vasculature wherein organ-specific angiocrine signals exert a vital role in maintaining physiologic tissue homeostasis and resolving pathologic challenges such as cancer and inflammation. Now, nearly a decade since pioneering this bold concept, the elucidation of angiocrine signaling mechanisms has taken center stage in vascular biology research, essentially affecting all organ disciplines and, most recently, also heavily impacting aging research.

In the context of tumor progression and metastasis, several unambiguous pieces of evidence highlight the central roles of endothelial cells as an instructive component within the metastatic niche. Involving multicellular cross-talk mechanisms, they orchestrate a conducive niche to allow single-seeded tumor cells to colonize a distant site. Following an overview of our current understanding of angiocrine

[1]European Center for Angioscience (ECAS), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany. [2]Division of Vascular Oncology and Metastasis, German Cancer Research Center Heidelberg (DKFZ-ZMBH Alliance), Heidelberg, Germany. [3]Faculty of Biosciences, Heidelberg University, Heidelberg, Germany. [4]German Cancer Consortium, Heidelberg, Germany.

Corresponding Authors: Mahak Singhal, European Center for Angioscience, Medical Faculty Mannheim, Heidelberg University, and German Cancer Research Center Heidelberg, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany. Phone: 4962-2142-1508; E-mail: m.singhal@dkfz.de; and Hellmut G. Augustin, European Center for Angioscience, Medical Faculty Mannheim, Heidelberg University, and German Cancer Research Center Heidelberg, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany. Phone: 4962-2142-1500; E-mail: augustin@angiogenese.de

Cancer Res 2020;80:659–62

doi: 10.1158/0008-5472.CAN-19-3351

signals in metastasis, this perspectives article will aim to identify key unanswered questions and discuss possible avenues to translate this novel knowledge into future therapeutic applications.

Vascular niches house and nourish disseminated tumor cells

The unique anatomic position of blood vessels necessitates a circulating tumor cell to closely interact with endothelial cells to arrive, home, and eventually survive at a metastatic site. Indeed, disseminated tumor cells may stay dormant in specialized vascular niches for long periods of time, possibly years, prior to giving rise to metastases. Latent breast cancer cells frequently reside in bone and brain microvascular niches where endothelial cells, by enhanced expression of tumor-suppressive TSP1, have been reported to promote sustained senescence of seeded tumor cells (1). Intriguingly, while the secretome of the resting mature vasculature maintained tumor cells in a quiescent state, activated endothelium conferred a tumor-promoting signature to facilitate metastatic growth. Likewise, the resting lung vasculature has been shown to express DARC, which is capable of inducing senescence in prostate cancer cells by physically interacting with KAI1 on tumor cells (2). Concurrently, gene expression of both DARC and KAI1 has been negatively associated with metastatic progression of different human cancers. It can be assumed that the resting vasculature imposes molecular checkpoints to resist metastatic growth and to maintain functional tissue homeostasis.

Besides providing an adhesive surface, the bone vasculature has recently been shown to release instructive signals for simultaneous induction of mesenchymal-to-epithelial transition and stemness of disseminated tumor cells (3). Mechanistically, direct engagement of endothelial E-selectin activated Wnt signaling in seeded breast cancer cells and induced cancer stem cell traits in a SOX2/9-dependent manner thereby enabling metastatic colonization. Genetic inactivation of E-selectin strongly suppressed bone metastases, but failed to affect lung metastasis, suggesting an organ-specific vascular control mechanism of metastatic progression. Similarly, activated endothelial cells formed a maladaptive niche in B-cell lymphoma cells, in which endothelial cell–derived JAG1 induced Notch signaling (4). Consequently, tumor cells acquired an invasive phenotype and produced FGF4, which enticed endothelial cells in a feedforward loop to further upregulate JAG1 production. Indeed, JAG1-NOTCH2–activated lymphoma cells displayed enhanced extranodal invasion and resistance to chemotherapy. Uncovering similar endothelial cell–derived factors will allow to better understand the rate-limiting steps of the metastatic cascade and to bolster the host defense against a metastasizing tumor.

Angiocrine signals orchestrate a protumor immune microenvironment

Blood vessels physically separate the circulation and the different parenchymal organ environments to form a tightly regulated interface allowing the selective infiltration of immune cells. Endothelial cells have long been known to mitigate immune cell adhesion and transmigration and to orchestrate cytokine amplification during viral infection and wound healing. Quiescent endothelium, either upon inflammatory stimulation or in the presence of tumor cells, was reported to rapidly release cytokines such as angiopoietin-2 (ANG2) to switch to an activated proinflammatory state. Autocrine-acting ANG2, in turn, induced STAT3 signaling in metastatic site endothelial cells, which resulted in enhanced expression of the chemoattractant CCL2 and the adhesion molecule ICAM1 (5). Subsequently, CCR2-positive macrophages were recruited to the metastatic site and manifested an immunosuppressive milieu to favor the survival of disseminated tumor cells. Consistently, endothelial-specific deletion of STAT3 or administration of CCL2-neutralizing antibody suppressed metastatic outgrowth in an angiogenesis-independent manner (5, 6), thereby underlining a critical role of the vasculature in choreographing the metastatic immune landscape.

Metastatic site endothelial cells with sustained activation of NOTCH1 signaling have been described to exhibit a senescent phenotype characterized by the overexpression of a wide-array of chemokines and the adhesion molecule VCAM1 (7). These molecular changes promoted the infiltration of myeloid-derived suppressor cells into the metastatic niche and facilitated postsurgical metastasis. Concurrently, mice preconditioned with NOTCH1-blocking antibody displayed reduced metastatic burden in experimental metastasis assays. Conversely, endothelial cells express nonsignaling chemokine-scavenging receptor DARC, which acts as a chemokine sink, thereby buffering potential surges in plasma chemokine levels. During tumor progression, endothelial cells overexpressed DARC to suppress chemokine bioavailability to eventually subside the host inflammatory response.

Endothelial cells and hematopoietic cells share a common cellular ancestry during development, called the "hemogenic endothelium." Secretion of cytokines such as SCF and CXCL12 as well as the expression of adhesion molecules by vascular niche cells, such as E-selectin, are essential for maintenance and retention of quiescent HSC within the bone marrow. In contrast, endothelial cells act as a TLR4-mediated sensor for pathogenic signals and promote emergency granulopoiesis to resolve a bacterial infection (8). Hence, endothelial cells exert functional control over both, steady state and pathogenic hematopoiesis. It is therefore not far-fetched that endothelial programs may be confiscated during metastatic progression to amplify systemic inflammation and suppress host defense mechanisms.

Mechanism-guided combination of vascular-targeted therapies with clinical standard care

VEGF/VEGFR-targeting drugs effectively block sprouting angiogenesis and leave behind a normalized intratumoral vascular network. Beyond their transient antiangiogenic effects, treatment with VEGF/VEGFR inhibitors induces an immune stimulatory milieu by enhancing the trafficking of cytotoxic T cells and repolarization of tumor-associated macrophages, suggesting a possible synergy with the immune checkpoint (IC) therapies. Indeed, cotargeting antiangiogenic and IC therapies has been shown to elicit an increased count of intratumoral high endothelial venules for selective leukocyte infiltration, thereby transforming an immunosuppressive to an immunosensitive tumor microenvironment (9). Similarly, antiangiogenic therapy could induce PD-L1 expression in otherwise PD-L1–negative tumors, rendering them susceptible to IC therapy.

ANG2, intensely pursued as a second-generation antiangiogenic target, was found upregulated in the serum of patients with metastatic melanoma. Intriguingly, serum ANG2 levels were inversely correlated with the overall response rate to IC therapy, indicating serum ANG2 as a prognostic biomarker for therapy outcome in patients with melanoma. Concurrently, a bispecific antibody targeting VEGFA and ANG2 could potentiate anti-PD1 treatment by functionally reprograming the immune landscape to provide sustained antitumor immunity (10). Similar to IC therapies, combining low dose metronomic chemotherapy and ANG2 neutralization strongly suppressed metastasis by limiting the recruitment of bone marrow–derived immunosuppressive myeloid cells and prolonged overall survival (5).

While the VEGF/VEGFR and the ANG/TIE signaling pathways have long been known to be crucial for neoangiogenesis, downregulation of TGFβ signaling has recently been identified as a key mechanism of establishing and maintaining vascular quiescence (11). Indeed,

metastatic progression involves the activation of the endothelium, which is associated with increased expression of ALK1, an endothelial-specific type 1 TGFβ receptor. Remarkably, ALK1-targeting agents stabilized endothelial cell junctions and suppressed metastatic dissemination of breast cancer (12). Furthermore, ALK1-targeting in conjugation with chemotherapy resulted in a long-term survival advantage in preclinical mouse models. Aberrantly activated endothelial cells downregulate IGFBP7, a tumor-suppressive checkpoint (13). Loss of IGFBP7 instigated stem cell–like traits in tumor cells and manifested chemoresistance, thereby assisting metastatic invasion in distant organ sites. Conversely, systemic administration of IGFBP7 reversed the maladapted vascular niche and sensitized tumor cells to chemotherapy. Overall, vascular-targeted therapies hold the potential to improve responsiveness and efficacy of current standard of care for oncology including chemotherapy and immunotherapy.

Future Challenges and Perspectives

The shift in preclinical oncology from fundamental discovery research to more translational therapy-oriented research and the emergence of metastasis as the most deadly hallmark of cancer require the adoption of a holistic approach beyond the narrower tumor cell–centric view to appreciate metastatic progression as a result of complex bidirectional interactions with the host microenvironment. Understanding the multicellular nature of the signaling cross-talk within the metastatic niche will accelerate the development of novel mechanism–based therapies. Indeed, tumor cells that may account for the bulk of the primary tumor are oftentimes the minor cellular constituent in the metastatic microenvironment heavily relying on stroma-derived signals for their survival and colonization. While vascular-targeted therapies have been extensively employed for their antiangiogenic effects, we are just beginning to appreciate tumor-promoting angiocrine functions of the vasculature and the therapeutic potential that could emerge from targeting them, most notably to restrict metastatic progression. Future translational research could well benefit from a better understanding of angiocrine signaling mechanisms during metastatic progression.

Establishing a systems map of evolving metastatic niches

Vascular niches support metastatic growth of disseminated tumor cells in an instructive manner either directly by imposition of molecular checkpoints or indirectly by orchestration of the stroma. Deconvoluting the underlying molecular signatures and establishing a dynamic multicellular systems map of the metastatic niche is an ambitious but achievable goal to expand our limited knowledge of the premetastatic and metastatic niches. Adoption of the latest sequencing technologies including single-cell RNA sequencing, spatial transcriptomics, and utilization of advanced postsurgical metastasis models will enable the realization of this ambitious goal. Such a comprehensive systems biology–based database may serve as a rich resource for future metastasis research and will serve as a reference to decipher the spatial and temporal evolution of the metastatic niche. It will furthermore allow to elucidate the role of angiocrine signals, beyond angiogenesis, in shaping the metastatic niche and will shed novel mechanistic insights for future therapeutic exploitation.

Elucidating organotypic endothelial response programs to tumor progression

Organotypic vascular differentiation and its gatekeeping functions on tissue homeostasis have recently been discovered in multiple organs. Yet, the responsiveness and functional influence of different vascular beds on metastatic progression remain poorly understood. While the organ tropism of a disseminated tumor cell is frequently described to be driven by cell intrinsic characteristics, it is still ambiguous how different vascular beds respond to the arrival of circulating tumor cells. Defining organ-specific angio-signatures will allow differentiating between locally defined metastatic niche-specific versus body-wide multiorgan endothelial response programs toward tumor progression. While the former will facilitate the identification of niche-specific therapeutic targets, the latter may uncover novel predictive biomarkers for tracing postsurgical metastatic progression. Indeed, an angiocrine factor–based biomarker may solve one of the major clinical hurdles, and allow to reliably stratify patients with metastatic cancer for stroma-targeted therapies, including immunotherapy.

Identifying mechanism-driven antimetastatic combinatorial therapies

IC therapies have revolutionized clinical oncology and, for the first time, offered in a hitherto unprecedented manner cure for patients with metastatic disease. However, the clinical benefit is limited to a subcohort of patients and the majority of patients (>85%) continue to be nonresponders. To improve response rates, multiple preclinical studies have demonstrated a strong synergy between antiangiogenic (anti-VEGF and anti-ANG2) and IC therapies. Indeed, ongoing clinical trials aim to assess the response rates and long-term efficacy of combining antiangiogenic and IC agents. As such, a major research focus has been on combining already approved drugs. However, research in the past decade has unveiled several novel angiocrine molecules (such as ALK1, IGFBP7, and JAG1), which exhibit profound antimetastatic effects in preclinical settings.

In conclusion, tumor–vessel interaction research has in the last decade substantially moved beyond the study of tumor angiogenic processes. The concepts of angiocrine signal transduction have enormously advanced vascular biology research. Translating these concepts into the field of tumor biology may and will greatly advance the mechanistic understanding of metastasis as being the result of complex multi-directional interactions between tumor cells and host stromal and immune cells. As spelled out in this perspective article, these lines of research also hold substantial potential to pave the way toward therapeutic approaches and, most notably, for laying a mechanism-based foundation for novel combination therapies to treat metastatic disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M. Singhal, H.G. Augustin
Writing, review, and/or revision of the manuscript: M. Singhal, H.G. Augustin
Study supervision: M. Singhal, H.G. Augustin

Acknowledgments

The authors regret that, owing to space limitations, they could not cite all original research articles and related references on this topic. Work in the authors' laboratory is supported by funds from the Deutsche Forschungsgemeinschaft (DFG), the European Research Council (ERC), and the Helmholtz Association.

Received October 24, 2019; revised December 2, 2019; accepted December 5, 2019; published first December 12, 2019.

References

1. Ghajar CM, Peinado H, Mori H, Matei IR, Evason KJ, Brazier H, et al. The perivascular niche regulates breast tumour dormancy. Nat Cell Biol 2013;15: 807–17.
2. Bandyopadhyay S, Zhan R, Chaudhuri A, Watabe M, Pai SK, Hirota S, et al. Interaction of KAI1 on tumor cells with DARC on vascular endothelium leads to metastasis suppression. Nat Med 2006;12:933–8.
3. Esposito M, Mondal N, Greco TM, Wei Y, Spadazzi C, Lin SC, et al. Bone vascular niche E-selectin induces mesenchymal-epithelial transition and Wnt activation in cancer cells to promote bone metastasis. Nat Cell Biol 2019;21:627–39.
4. Cao Z, Ding BS, Guo P, Lee SB, Butler JM, Casey SC, et al. Angiocrine factors deployed by tumor vascular niche induce B cell lymphoma invasiveness and chemoresistance. Cancer Cell 2014;25:350–65.
5. Srivastava K, Hu J, Korn C, Savant S, Teichert M, Kapel SS, et al. Postsurgical adjuvant tumor therapy by combining anti-angiopoietin-2 and metronomic chemotherapy limits metastatic growth. Cancer Cell 2014;26:880–95.
6. Kim KJ, Kwon SH, Yun JH, Jeong HS, Kim HR, Lee EH, et al. STAT3 activation in endothelial cells is important for tumor metastasis via increased cell adhesion molecule expression. Oncogene 2017;36:5445–59.
7. Wieland E, Rodriguez-Vita J, Liebler SS, Mogler C, Moll I, Herberich SE, et al. Endothelial Notch1 activity facilitates metastasis. Cancer Cell 2017;31:355–67.
8. Boettcher S, Gerosa RC, Radpour R, Bauer J, Ampenberger F, Heikenwalder M, et al. Endothelial cells translate pathogen signals into G-CSF-driven emergency granulopoiesis. Blood 2014;124:1393–403.
9. Allen E, Jabouille A, Rivera LB, Lodewijckx I, Missiaen R, Steri V, et al. Combined antiangiogenic and anti-PD-L1 therapy stimulates tumor immunity through HEV formation. Sci Transl Med 2017;9:eaak9679.
10. Schmittnaegel M, Rigamonti N, Kadioglu E, Cassara A, Wyser Rmili C, Kiialainen A, et al. Dual angiopoietin-2 and VEGFA inhibition elicits antitumor immunity that is enhanced by PD-1 checkpoint blockade. Sci Transl Med 2017;9: eaak9670.
11. Schlereth K, Weichenhan D, Bauer T, Heumann T, Giannakouri E, Lipka D, et al. The transcriptomic and epigenetic map of vascular quiescence in the continuous lung endothelium. Elife 2018;7:e34423.
12. Cunha SI, Bocci M, Lovrot J, Eleftheriou N, Roswall P, Cordero E, et al. Endothelial ALK1 Is a therapeutic target to block metastatic dissemination of breast cancer. Cancer Res 2015;75:2445–56.
13. Cao Z, Scandura JM, Inghirami GG, Shido K, Ding BS, Rafii S. Molecular checkpoint decisions made by subverted vascular niche transform indolent tumor cells into chemoresistant cancer stem cells. Cancer Cell 2017;31: 110–26.