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Abstract

It has been 40 years since Folkman's seminal paper [Cancer Res 1974. 34:2109-13], proposing the presence of a tumour associated angiogenic factor, which could be targeted as an anticancer therapy. There are currently a handful of drugs in trial or use that have been marketed as targeting angiogenesis. Unfortunately, the most widely used of these, bevacizumab (Avastin™, Roche), has met with limited success clinically. For this reason and based on a calculation of cost benefit, bevacizumab is now only publically subsidised for use in a limited range of solid tumours. That the contribution of vasculature to malignancy remains poorly understood is increasingly clear. At the same time, the traditional view that vascularisation is a passive participant in the process of malignancy, and that endothelium merely provides a conduit by which tumour cells spread, is being replaced with an understanding that vasculature is a key player in the process of metastasis. Furthermore, the identification of non-traditional sources of vasculature has complicated our understanding of the tumour endothelium as a unique population that can be simply targeted as an anticancer therapy. The following review seeks to provide an up-to-date view of vascular contribution to metastasis and implications for new vasculature-targeted anticancer treatments.

At face value, the vascular contribution to metastasis is not self-evident. Many lesions that a patient may present with are vascular, despite being poorly malignant or benign. Consequently, the relationship between vascularity, tumour growth and malignancy remains controversial. In many instances, tumours can present with metastatic lesions, while the primary lesion remains relatively small, or undetected. Conversely, relatively large and highly vascular breast lumps can also remain undetected and benign for many years. Yet despite these observations, vascularisation and spread are closely linked with clinical course in many solid tumours, including breast cancer.

While benign tumours can either be vascular or relatively avascular, almost all malignant tumours are vascular. Furthermore, while distal lymph node metastasis remains the main pathological indicator of grade, it is increasingly clear that spread via the lymphatics is a later event in metastasis. This is supported by the detection of disseminated tumour cells in the peripheral blood and bone marrow of patients with early stage breast cancer. Haematologic dissemination of tumour cells may therefore be considered the initial route for early spread of the tumour, although evidence for this to date remains circumstantial. Regardless, because of its important role in cancer spread, the role that vasculature plays in establishment and growth of metastasis at a secondary site has occupied a lot of research effort. It has been known for several years that drugs targeting vascular endothelial growth factor (VEGF) can enhance the anti-tumour effects of cytotoxic drugs. While the mechanism for this is uncertain, it is proposed that the effectiveness of combination therapies slows angiogenesis, leading to a normalisation of the vascular tree, and enhanced drug delivery. However, many of these therapies are associated with the onset of adaptive resistance (a loss of response to therapy), as well as a tumour environment that is selective for the development of highly aggressive tumour cells.

Understanding the role that vasculature plays in malignancy is key to the effective use of current therapies, and in the development of future effective therapies. To date, despite a sustained research effort over many years, only a handful of anti-angiogenic drugs are in clinical use.

Anti-angiogenic therapy

The Food and Drug Administration (FDA) in the US has approved several drugs that have antiangiogenic activity, including bevacizumab (Avastin®). Bevacizumab is the most widely used antiangiogenic therapy. It is designed to stop VEGF signalling and thus vascular growth. The FDA has approved bevacizumab to be used alone for, glioblastoma and in combination with other drugs for the treatment of: (i) metastatic colorectal cancer; (ii) non-small cell lung cancers; and (iii) renal cell cancer. Alone, bevacizumab is ineffective for the treatment of certain cancers, or is effective for a very short period of time as a result of adaptive resistance. When combined with traditional chemotherapy (such as FOLFOX or FOLFIRI), bevacizumab provides colorectal cancer patients an average increase in survival of five months. Patients with advanced non-small-cell lung cancer have an average increase in survival of about two months. These increases in survival in patients with highly advanced disease are significant, and it should be emphasised that they may translate into longer individual survival expectations when used in the actual target group.

The effectiveness of bevacizumab treatment in breast cancer has been more controversial. Although phase III trials of bevacizumab plus chemotherapy demonstrate modest effectiveness in metastatic breast cancer,
these trials used progression free survival as a measure of efficacy. However, breast cancer populations are heterogeneous, and contain rare cell populations that are undetectable and capable of proliferating aggressively regardless of tumour size. In contrast, clinical trials using overall survival fail to show clinical advantage to the use of bevacizumab. Furthermore, a significant proportion (2.5%) of patients on bevacizumab therapy will experience a fatal adverse event, resulting from haemorrhage. These include neutropenia with lethal infection, gastrointestinal tract perforation, pulmonary embolism and cardiovascular accident. There are also a range of side-effects associated with bevacizumab therapy, including hypertension, cardiac toxicity, neutropenia, thromboembolisms, stroke, enhanced chemotherapy toxicity and impaired wound healing resulting in severe bleeding. Finally, bevacizumab doubles the cost of chemotherapy to $100,000 USD per patient per year. For these reasons, as of December 2010, after only two years of use, the FDA withdrew bevacizumab treatment for metastatic breast cancer. The Therapeutic Goods Administration in Australia, and the National Health Service in the United Kingdom, have followed suit and do not provide government funding for bevacizumab treatment of malignant breast cancer.

Given the lack of effectiveness of bevacizumab in advanced breast cancer, but the clear advantage in other types of cancer, there remains a need for improved anti-angiogenic therapies, as well as the need to obtain a greater understanding of the underlying mechanisms supporting breast cancer angiogenesis, resistance and progression.

**Mechanisms of neovascularisation**

The classical model for tumour neovascularisation proposes that as a solid tumour grows, it first starts by co-opting pre-existing vessels, without vasculogenesis (de novo generation of vessels), and by recruiting pre-existing endothelial cells from surrounding tissues (angiogenesis) (figure 1). The transition from an avascular adenoma to a fully vascular, metastasising lesion, is referred to as the angiogenic switch, and its onset is associated with rapid tumour growth. Fifteen years ago, Asahara put forward a further mechanism by which tumours recruited vasculature. He proposed that a certain proportion of tumour vasculature was derived de novo from bone marrow adult stem cells. Since then, bone marrow derived endothelial progenitor cells (EPCs), with vasculogenic potential and capable of luminal incorporation into the vascular tree, have been identified as an alternate source of tumour vasculature.

More recently, several post-classical mechanisms of neovascularisation have come to light, including tumour cells themselves, myeloid cells and tissue resident mesenchymal stem cells (MSCs). All of these populations have been found to acquire vascular markers and mimic endothelial cell biology, and therefore must be considered in the development of anti-angiogenic therapies. However, the role of vasculogenesis and vascular mimicry in the adult remains controversial because of the numbers of cells implicated in tumour pathology, and because of limitations in the tools available to study them.

**Vessel co-option**

In vessel co-option, neither vasculogenesis nor angiogenesis play a role. Instead, the growing tumour simply incorporates existing vasculature from the host tissue bed. Studies suggest that vessel co-option occurs in the initial stages of tumour growth. Notably, tumour regrowth following anti-angiogenic therapy commonly involves vessel co-option, in addition to both angiogenesis and vasculogenesis.

**Angiogenesis**

Angiogenesis refers to the formation of new blood vessels from pre-existing vessels, and occurs concurrently with vasculogenesis in a rapidly vascularising tumour. During angiogenesis, hypoxia drives vessel sprouting via VEGF mediated proliferation of endothelial cells, and basement membrane remodelling. Sprouts invade surrounding tissues as a proliferating solid stalk, which is guided by a ‘tip cell’, possessing numerous filopodia that sense gradients of angiogenic molecules. Formation of a functional lumen and integration into existing vascular structures follow. As a consequence, sprouting angiogenesis is slow to develop an organised structure and is dependent upon cell proliferation, as well as the activation of molecular pathways that support tissue invasion. Vessels formed in this way tend to be disorganised, leaky and disconnected from the existing vascular tree.

Distinct from sprouting angiogenesis, intussusceptive angiogenesis is characterised by the formation of...
transvascular tissue pillars across the vessel lumen.\textsuperscript{26} Vessel walls join together, leading to the formation of a transverse endothelial blayer, or pillar. The pillar then undergoes perforation, and dilatation. This is followed by branching and further development, including invasion of fibroblasts and pericytes, as well as the laying down of interstitial matrix.\textsuperscript{27} As a consequence, intussusceptive angiogenesis leads to rapid neovascular branching (occurring in hours or even minutes), and does not require cell division. Once started, intussusception can dramatically increase vascular surface area, and unlike sprouting, allows for continuous blood during vessel formation. Intussusceptive angiogenesis has been demonstrated in a range of cancers, and is linked to regression following anti-cancer therapy.\textsuperscript{28,29} While no single molecule has been implicated as a driver of intussusception, stress has been shown to play a role.\textsuperscript{30}

**Vasculogenesis**

In embryology, vasculogenesis refers to the de novo formation of blood vessels from the differentiation of mesodermal precursor cells, referred to as endothelial precursor cells (EPCs).\textsuperscript{31} There are two main theories for the origins of embryonic EPCs: (i) the idea that there exists a bipotential haemangioblast that comes directly from mesoderm and forms both the early vasculature as well as the haematopoietic system, and (ii) the proposition that there exists haemogenic endothelium (haemangioblast), an endothelial cell intermediate with haematopoietic potential which is not derived directly from mesoderm.\textsuperscript{32-34} While studies suggest that the haemangioblast is the source of both haematopoietic cells and the majority of endothelium in embryos, the haemangioblast in adults remains undefined.

EPCs can collect both in normal and cancerous tissues, and contribute to de novo blood vessel growth.\textsuperscript{13-16,35} Since they were proposed, observations have shown that circulating EPCs contribute to tumour angiogenesis.\textsuperscript{14,36} However, because of their small number and because circulating vasculature shed from the tumours expresses many markers that are similar to EPCs, consensus as to their importance in human cancer biology has been difficult to arrive at.\textsuperscript{37} In 2005, Peters et al demonstrated conclusively that EPCs were present in human cancer.\textsuperscript{13} This was supported by studies in mice,\textsuperscript{14,16} which showed that EPCs could be tracked from the bone marrow to luminal incorporation into tumour vascular and that they were present at the tumour periphery in the early stages of the angiogenic switch. In 2008, Gao et al\textsuperscript{15} demonstrated that metastases also underwent an EPC dependent angiogenic switch. At the time, it was not self-evident that a metastasis, which had already undergone an angiogenic switch prior to spread, would have to undergo further changes to recruit vasculature to a secondary site. The fact that EPCs are not only participants in metastasis, but significant players in driving malignant spread, has made them important targets of anticancer therapy.

**Vascular mimicry**

In addition to EPCs, some aggressive cancers show a remarkable functional plasticity, exhibiting the phenomenon of vasculogenic, or vascular mimicry.\textsuperscript{17} During vascular mimicry, cancer cells and/or cells of a non-endothelial lineage begin to express genes associated with angiogenesis and vasculogenesis, assuming some phenotypic traits of endothelial cells, including luminal incorporation. Vascular mimicry is driven by tumour hypoxia, and tumours displaying vascular mimicry exhibit a matrix-rich, vasculogenic-like network, lined with transendothelial tumour cells that have assumed the function of endothelial cells.\textsuperscript{38} The earliest description of vascular mimicry was reported in melanoma by Maniotis,\textsuperscript{39} who described vascular-like networks of tubular and non-tubular structures, which were rich in collagen, possessing a basement membrane lined with tumour cells co-expressing endothelial markers, and containing plasma and red blood cells. Further work has demonstrated similar structures in a range of aggressive tumour types, including carcinomas, sarcomas, glioblastomas and astrocytomas.\textsuperscript{40-42} Tumour cells displaying vascular mimicry show phenotypic plasticity similar to embryonic stem cells, expressing key stem cell markers,\textsuperscript{43} as well as expression of endothelial markers such as VE-cadherin, erythropoietin-producing hepatocellular carcinoma-A2, and extracellular matrix proteins (fibrinogen and collagen IV and VI), and down-regulation of genes that are cancer/epithelial cell specific.\textsuperscript{44}

Functionally, the leaky vessels created via vascular mimicry provide an alternate perfusion route. Furthermore, channels of vascular mimicry may also connect to vessels, increasing the overall perfusion of the tumour, as well as providing a pathway for metastasis. Clinically vascular mimicry, although rare, is associated with poor prognosis, suggesting that it confers an advantage in tumour progression. Furthermore, as tumour cells displaying vascular mimicry lack the regulatory constraints on growth and differentiation displayed by normal endothelial cells, and are genetically unstable, they would be subject to the same propensity that cancer cells have to develop drug resistance.

In addition to tumour cells, there is a growing recognition that host derived cells with nonvascular lineages may also begin to express markers normally associated with endothelial cells. By far the best-studied bone marrow-derived tumour infiltrating cells contributing to angiogenesis, which may be candidates for myeloid vascular mimicry, are tumour-associated macrophages (TAMs).\textsuperscript{45} As with EPCs, TAMs are recruited in response to tumour derived chemokines and growth factors.\textsuperscript{46} TAMs produce pro-angiogenic molecules VEGF, interleukin-8, tumour necrosis factor-α and matrix metalloproteinase-9 (MMP-9).\textsuperscript{47} Correspondingly, high numbers of TAMs often correlate with tumour vascularisation.\textsuperscript{18,48} In addition, there is increasing evidence that TAMs start to express endothelial factors such as CD31 and they may contribute to tumour vasculature directly, although there is little evidence of luminal incorporation as endothelium and their role may be solely perivascular.

MSCs are found in many tissues, including bone marrow, and represent another heterogeneous stromal cell lineage that has been implicated in tumour angiogenesis and growth. Tissue resident MSCs play a role in the maintenance and regeneration of connective tissues through engraftment.\textsuperscript{49,50} During cancer, significant
numbers of MSCs are recruited to the site of the primary tumour, where they play a role in invasion, metastasis and immunological evasion.\(^3\),\(^2\) Non-bone marrow derived MSCs have recently been proposed as a source of vasculature in certain tumours.\(^5\)

**Dormancy and the endothelium**

Studies in breast cancer metastasis have shown that dormant disseminated tumour cells reside within the lumen of microvasculature at common metastatic destinations such as the lung, bone marrow and brain.\(^3\) Three-dimensional modelling of microvasculature in vitro has confirmed that the perivascular location of tumour cells is responsible for maintaining their quiescent state. It has also been shown that the protein thrombospondin-1 is secreted by endothelial cells, and can act to suppress tumour-cell growth at a secondary site.\(^3\) Remarkably, this growth-suppressive microenvironment is found only around stable vasculature. Activated or sprouting tips of newly forming vessels actually have a growth-accelerating effect on tumour cells, through expression of pro-metastatic proteins, including periostin, tenascin-C, fibronectin and tumour growth factor-\(\beta1\). Recent work by Wells et al. (University of Pittsburgh Medical Centre, US) has shown that transformed endothelial cells from the liver also confer a proliferative advantage to breast cancer cells, via the epidermal growth factor pathway.\(^6\) While the role of endothelial cells in maintaining dormancy has yet to be shown in vivo, emerging lines of evidence such as these are compelling and provide the first tantalising glimpse of the importance of the vascular tree in regulating growth of secondary metastases.

**Conclusion**

Tumour neo-vascularisation is complex, involving both interlinked and distinct processes. Recruitment of pre-existing vasculature is characterised by complex architectural changes to the existing vascular tree. Furthermore, our lab and others,\(^14\)-\(^16\) have shown that EPCs and other bone marrow derived cells are significant drivers of angiogenesis and spread, and may be the main factor underlying development of adaptive resistance. It is also evident that other vasculogenic populations such as tumour cells themselves and cells of non-bone marrow/ non-endothelial origin, are also important drivers of clinical course in cancer. The understanding that endothelial cells at the site of secondary metastasis can regulate growth and further spread directly, is a new revelation in cancer biology and has led to a greater appreciation of the role of the vasculature as drivers of malignancy and drug resistance. Targeting tumour angiogenesis is increasingly being used in anticancer strategies. These therapies are attractive, as endothelial cells from the body are not subject to the same selective pressure or genetic instability as tumour cells, and may be less likely to develop resistance. However, delivery of anti-angiogenic therapy treatment must be targeted and based on an understanding of the processes that underlie tumour vascular biology.

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**References**

That cancer progression relies heavily on the interaction between malignant cells and host stromal cells within the tumour microenvironment, including fibroblasts, endothelial cells and immune cells, is now well accepted. Although initially thought to have anti-tumour roles, it is now well known that immune cells can also become influenced by tumour-derived and other microenvironmental factors and adopt a pro-tumorigenic, immunosuppressive phenotype. In the last three decades, much attention has been focused on the roles of myeloid-derived suppressor cells (MDSCs) in this process. Mostly known for their ability to suppress the anti-tumour immune response through modulation of T cells, natural killer cells, dendritic cells and macrophages, as evidenced by tumour-derived and other microenvironmental factors and adopt a pro-tumorigenic, immunosuppressive phenotype. In the last three decades, much attention has been focused on the roles of myeloid-derived suppressor cells (MDSCs) in this process. Mostly known for their ability to suppress the anti-tumour immune response through...