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Current Controversies in Cancer

Is Determination of Angiogenic Activity in Human Tumours Clinically Useful?

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PIONEERING RESEARCH from the laboratory of Dr Judah Folkman has established that angiogenesis is essential for primary and metastatic tumour growth [1, 2]. The concept that angiogenesis is critical in both physiological and pathological processes has led to an overwhelming number of investigations in this field: a recent MEDLINE search revealed more than 800 papers per year on angiogenesis [3]. The goal of the research is to eventually improve patient care. Admittedly, how the role of angiogenesis will ultimately affect the care of the cancer patient is at present undefined. However, the rapid acquisition of knowledge in this field should provide the oncologist with a strong foundation with which to determine how to use tumour angiogenesis as a diagnostic tool and even, perhaps, as a novel therapeutic target. The 'pro' position on the topic of 'Is determination of angiogenic activity in human tumours clinically useful?' is not written from the perspective of the *current* clinical utility of knowledge about tumour angiogenic activity, but rather the *potential* importance of tumour angiogenic activity in the care of the cancer patient after more thorough investigations are completed in appropriately designed prospective clinical trials.

Although, prior to this decade, there were rare reports of the association of neovascularity and staging or prognosis, it was not until the publication of an article in 1991 by Weidner and associates that the potential role of vessel counts as a prognostic factor was recognised [4–6]. Since that publication, the role of angiogenesis as a prognostic factor has been examined in more than 20 studies on breast cancer and nearly every other solid tumour type (reviewed in [7]). Although most studies demonstrate a correlation between an increase in microvessel density and either metastasis formation, later stage of disease, or poorer prognosis, not all studies concur (discussed below).

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The potential role of angiogenic activity in the clinical care of the cancer patient can essentially be divided into two major categories: (1) as a prognostic factor or tumour marker; and (2) therapy. Tumour microvessel density or angiogenic factor expression may predict which patients are likely to develop metastasis. Serum levels of angiogenic factors could serve as tumour markers. The second major goal of determining angiogenic activity in tumours is to select patients with highly angiogenic tumours that might benefit from treatment with anti-angiogenic therapy.

In the absence of prospective trials, it is premature to assess the clinical value of tumour angiogenic activity. However, retrospective clinical studies and experimental evidence indicate that tumour angiogenesis is likely to play pivotal roles in understanding the biology of metastasis, determining the prognosis of patients and developing novel therapeutic strategies.

THEORETICAL ISSUES

The association between tumour angiogenesis and metastasis is based on two principles [6]. The first is that the more angiogenic a tumour is, the greater its capacity to increase in size. It has been demonstrated that the number of circulating tumour cells shed from a primary tumour is a direct function of the size of a primary tumour, i.e. the cellular mass [8]. The greater the number of cells shed in the circulation should theoretically lead to a greater number of cells which have the capacity to form a clinically relevant metastasis [8, 9]. The second reason angiogenic activity may correlate with an increase in metastasis is that there is a larger surface area over which tumour cells and the vasculature may interact, thus allowing a greater probability of haematogenous dissemination. Since most patients with solid malignancies die of their metastatic disease and most metastases are refractory to standard antineoplastic therapy, the ability to form metastases directly translates into decreased overall survival.

Most studies examining angiogenic activity in human tumours are based on utilising an immunohistochemical marker of the microvasculature of tumours that makes it possible to count the vessels within a specified area of the tumour. Numerous endothelial cell-specific antibodies have been utilised to highlight the neovasculature [10]. Once the neovasculature is highlighted, the vessel density must be assessed by one of several methods, either by counting vessels in a high powered field (as described by Weidner) with the assistance of image analysis, or by noting the number of instances that a grid overlies a vessel [10]. This variability in methodology and the potentially great variability in the patient population, tumour histopathology, tumour stage, treatment modalities and preservation of tumour specimens make it unsurprising that there are discrepancies in the literature regarding the prognostic value of vessel counts.

The role of angiogenesis in the metastatic cascade

It must be realised that in the process of metastasis, angiogenesis is only a single step among many. The process of metastasis is a highly selective and sequential process that requires alterations in gene expression so as to allow a tumour cell to proceed through each step (Figure 1) [11]. In other words, a highly angiogenic tumour must possess additional characteristics in order to proceed through the metastatic cascade and successfully form metastases. If a tumour cell fails to progress through any one of the steps, it cannot form a metastasis [12].

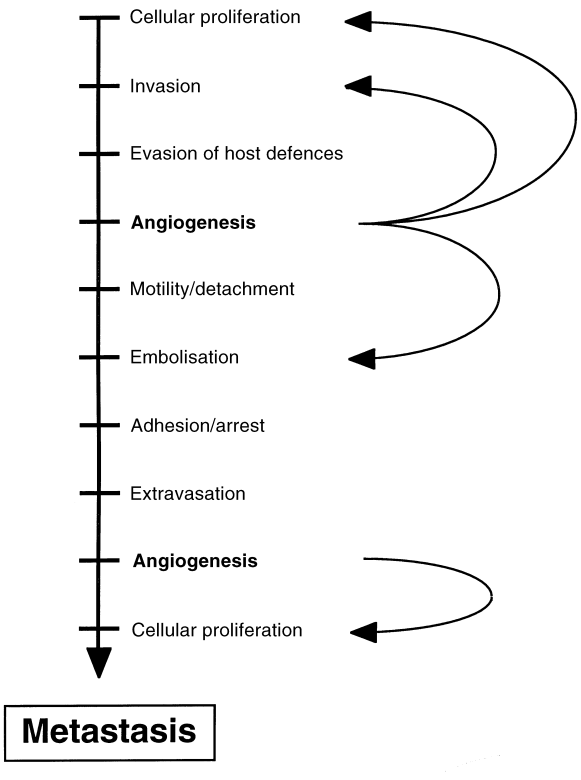

Figure 1. Steps in the process of metastasis. For a tumour cell to form a metastasis, it must express the appropriate genes which allow it to proceed through all the steps in the metastatic cascade. Angiogenesis is an essential step in the process, but by itself is not sufficient to allow a tumour cell to proceed through the entire metastatic cascade. However, angiogenesis is inherently involved in other steps of the metastatic cascade, including invasion, embolisation, and cell proliferation.

How is it that measurement of a single parameter (i.e. angiogenesis) of the metastatic cascade predicts metastasis formation? Once a tumour becomes clinically relevant (i.e. palpable, symptomatic, or detectable by imaging studies), it is likely that the tumour has already acquired the ability to proliferate, invade and establish its own blood supply to some degree. In addition, many of the factors that regulate angiogenesis also regulate tumour cell invasion (i.e. degradative enzymes, motility factors, etc.). Therefore, the clinically detectable tumour has a head start on the process of metastasis and only has to proceed through the last few steps of the metastatic cascade before it becomes a clinically relevant metastasis.

Theoretically, highly angiogenic tumours give access to a greater number of tumour cells that may enter the circulation than less angiogenic tumours. In addition, the greater the vessel density, the greater the chance that the vasculature will come into contact with cells that have the necessary phenotype to complete the metastatic cascade. The greater the number of tumour cells that enter the circulation, the greater the chance that a cell with the metastatic phenotype will proceed through all stages of the metastatic cascade. Furthermore, it is possible that a highly vascularised tumour may not only provide a nutrient blood supply to the tumour mass, but the newly formed endothelial cells may secrete factors that increase tumour cell growth directly in a paracrine manner [13, 14].

Angiogenesis (vessel density) as a biomarker

In an effort to obtain prognostic information by examining the various steps of the metastatic cascade, it has been necessary to look at gene expression for the factors that regulate each step. For example, to determine the ability of a tumour to invade the basement membrane or to be motile, one must investigate the relative expression of the factors that regulate these processes. It is impossible to perform a functional assay on these steps of the metastatic cascade in paraffin-embedded tumour samples and one can only guess which of the many redundant genes identified in regulating each step is important in any individual tumour. However, angiogenesis can be directly measured and so can serve as a biomarker [15]. The vessel density in a tumour is a direct measurement of the angiogenic activity. Although angiogenic factor expression has been shown to be a prognostic marker in certain tumour types, these factors are at least one notch upstream of the biologically important phenotypic expression of that factor [16–18].

Relationship of angiogenic activity to metastasis

In any study of the effect of angiogenic activity on prognosis or metastasis formation, the investigator may encounter a solid tumour that has a high angiogenic activity but is not associated with metastasis or decreased survival. This lack of correlation may be due to the inability of the tumour, although highly angiogenic, to progress through one or more of the other steps in the metastatic cascade. Occasionally, a tumour may be poorly angiogenic and yet may be associated with metastasis. *How can this be explained?* It is well known that tumours are heterogeneous in their phenotype and expression of particular genes. Although tumours may begin as a clonal outgrowth, as tumour cells rapidly divide they are more likely to acquire an increased number of mutations and thus become increasingly heterogeneous. Although mean

vessel density may be low (i.e. the average of several highly vascularised areas), one particular area of a tumour may have a high angiogenic activity, allowing the egress of tumour cells into the circulation at a high rate. Thus, this apparently 'low angiogenic' tumour may give rise to a clinically relevant metastasis. However, most tumours with a low angiogenic index are unlikely to form a clinically relevant metastasis because there is: (1) less surface area for tumour cell–vessel interface; and (2) a low probability that the tumour cell embolus will itself induce angiogenesis at a distant site. Perhaps the oncologist would gain more reliable information from the angiogenic index in those patients with *poorly* angiogenic tumours, which afford a better prognosis, than in those patients with highly angiogenic tumours, where the prognostic value is perhaps less reliable.

If an increase in angiogenesis is associated with an increase in metastasis formation, then it follows that an increase in angiogenic factor expression in tumours should be associated with an increase in metastasis. This principle is supported by experimental studies where tumour implants in mice of melanoma cells transfected with a vascular endothelial growth factor (VEGF) construct demonstrated an increase in the number of lung metastases compared with controls [19]. Alternatively, decreasing angiogenic factor activity (antibodies to VEGF) has been demonstrated to decrease metastasis formation in experimental models [20, 21].

Angiogenic factors as tumour markers

The development of the tumour neovasculature is dependent upon the balance of negative and positive regulators of angiogenesis [22]. Studies have demonstrated an association between tumour angiogenic activity and increased expression of specific angiogenic factors [23–27]. Investigators have sought to determine if these factors are increased in the serum of patients with solid tumours. Basic fibroblast growth factor (b-FGF) has been found to be elevated in the serum of patients with renal cell carcinoma, an increase that may be associated with advanced disease [28–30]. In two separate series, a small number of patients with breast or cervical cancer had elevation of serum b-FGF prior to clinical relapse [31, 32]. Serum b-FGF has also been shown to be elevated in patients with prostate cancer [33]. Other studies have shown b-FGF to be elevated in the urine of patients with a wide variety of tumours, an increase that was most pronounced in those patients with metastatic disease [34].

VEGF has also been examined in the serum of patients with cancer. In patients with rapidly growing colorectal cancer, serum VEGF and b-FGF are higher than in the serum of patients with slower growing tumours [35]. In patients with breast cancer, VEGF serum levels have been found to be higher in patients with advanced stage disease and larger tumours [36]. Following surgery, VEGF in the serum decreased in 5 of 6 patients, and of 6 patients in this series that relapsed, 3 had an increase in serum VEGF levels. In addition, serum VEGF levels correlated with the microvessel density of the primary tumour. Although no studies have compared standard tumour markers (e.g. carcinoembryonic antigen) to serum angiogenic factor levels, these preliminary findings suggest that further study is warranted to determine the clinical utility of these factors in the clinical care of the cancer patient.

CLINICAL ISSUES

How can clinically relevant information be obtained from tumour angiogenic activity?

In order to address the clinical value of tumour angiogenic activity, several issues must be examined. For a prognostic marker to be useful, three criteria must be obtained. First, does the prognostic marker improve the ability to predict recurrences compared to current prognostic markers? Second, can this marker be widely studied with reproducibility and quality control by oncologists practicing in different centres? Third, once we obtain information from such a marker, can we do anything with that information (for example, do we have effective therapies for a group of patients deemed to be at a high risk for recurrence?)?

The role of angiogenesis as a prognostic marker cannot be generalised to all tumour types. The power of any diagnostic test is optimised when the appropriate patient population is selected for study. For angiogenesis to be associated with metastasis in certain tumours, one must consider the role of angiogenesis in the patterns of growth and spread of such tumours. For example, tumour types that recur at distant sites and induce morbidity by their increasing tumour mass are more likely to be angiogenesis dependent than tumour types that recur locally by way of tumour 'seeds' or 'sheets' and produce morbidity by local infiltration. We have examined the significance of angiogenesis in intestinal-type and diffuse-type gastric cancer [26]. In intestinal-type gastric cancer, which tends to metastasise haematogenously to the liver, vessel density correlated with stage of disease. In contrast, in diffuse-type gastric cancer, which tends to recur locally as peritoneal metastases, vessel density did not correlate with stage of disease. Vessel density and VEGF expression were higher in intestinal-type disease than in diffuse-type disease, suggesting that recurrence in intestinal-type disease is more angiogenesis dependent. We have similarly found that vessel counts do not predict recurrences in pancreatic cancer, a disease that tends to recur loco-regionally (Ellis, data not shown). Thus, the value of vessel counts as a prognostic marker may be dependent on the tumour type under study and its pattern of failure. In tumours that tend to fail by way of peritoneal seeding or spread along tissue planes (such as meningiomas), tumour angiogenesis may not be as essential as in tumours with three-dimensional growth patterns.

Is the angiogenic index a better prognostic marker than standard markers?

Several studies utilising univariate and multivariate analyses in patients with node-negative breast cancer have demonstrated that vessel count is a strong prognostic factor. Vessel count in this group of patients may in fact be better than other standard prognostic factors, such as size, age, or hormone status [37–40]. In fact, in several series, vessel count has been shown to be a stronger prognostic factor than even nodal status [6, 41, 42].

The angiogenic index may not be the single best predictor of prognosis, but may be a component of a useful battery of prognostic factors. By *in situ* hybridisation, our laboratory has demonstrated that the expression of several factors implicated in metastasis (including b-FGF as an angiogenic factor) was more useful than any single factor in predicting metachronous distant metastasis in patients with node-negative colon cancer [43, 44]. Gasparini and colleagues found that vessel density and laminin-receptor expression were the strongest

prognostic markers in patients with node-negative breast cancer [45]. Thus, to improve prognostication, angiogenic activity should be viewed as one important component of a more comprehensive determination of factors or biomarkers that represent multiple steps in the metastatic cascade.

If patients present with highly angiogenic tumours and these are the patients most likely to develop distant recurrences, then it follows that this group of patients are most likely to benefit from adjuvant therapy. Few studies have examined this premise. Gasparini and associates have investigated the role of adjuvant hormonal or chemotherapy on survival in patients with node-positive breast cancer [46, 47]. However, patients with high tumour angiogenic activity did not appear to benefit from either form of adjuvant therapy. Therefore, in contrast to the above stated hypothesis that highly angiogenic tumours are more likely to recur and, thus, patients harbouring these tumours are more likely to benefit from adjuvant chemotherapy, these studies demonstrated that highly angiogenic tumours have a more aggressive phenotype and do not benefit as anticipated. It may be that patients with low angiogenic tumours are more likely to benefit from adjuvant therapy, even though they are also the subgroup of patients least likely to develop a recurrence. Patients with highly angiogenic tumours should perhaps be selected for adjuvant trials that include the use of anti-angiogenic strategies.

Is the measurement of angiogenic activity readily available and reproducible?

Discrepancies in studies investigating angiogenesis in solid tumours have shown the importance of methodology and patient selection in providing reproducible results. Immunohistochemistry is the mainstay of all pathology laboratories, and antibodies to numerous endothelial cell markers are easily accessible. However, there is such a wide range of antibodies (and suppliers), antigen retrieval methods, designation of high and low vessel count groups (cut-off points), patient study groups, therapies and data (vessel quantification) interpretation, that it is exceedingly difficult to compare results. In an effort to standardise the methodology for vessel staining and interpretation, leaders in the field of angiogenesis determination recently published guidelines [10]. Prospective studies using standardised techniques will better define the role of tumour angiogenic activity as a prognostic marker.

Can patients with highly angiogenic tumours benefit from antineoplastic therapies?

Determining which patients are at a high risk of failure is inconsequential to the patient if there are no effective therapies to treat either microscopic or clinically detectable disease. As noted above, adjuvant chemo- and/or hormonal therapy may not prove beneficial to the patient at high risk for recurrence based on the angiogenic activity of the tumour [46, 47]. It is possible that certain patients may benefit from adjuvant anti-angiogenesis therapy. In addition, if certain metastatic tumours are known to have high angiogenic activity, then these patients may also potentially benefit from anti-angiogenic therapy. It may be that the optimal benefit to patients is derived from a combination of anti-angiogenic therapy and standard systemic regimens.

The field of anti-angiogenesis is one of the most active areas of translational research. Table 1 lists anti-angiogenic agents that are currently under investigation or potentially may be examined in clinical trials. Prior to initiating and interpreting anti-angiogenic clinical trials, the clinical oncologist must be attuned to important differences from standard cytotoxic chemotherapeutic trials. First, anti-angiogenic therapy may need to be delivered on a chronic basis, since this type of therapy is not cytotoxic but rather only prevents further growth of a tumour. Therefore, therapy must be well tolerated with minimal untoward side-effects. Second, the end-point of anti-angiogenic therapy would not be tumour shrinkage but rather tumour stabilisation. This end-point is a new concept in cancer clinical trials: tumour stabilisation over a period of time should be considered a desirable event rather than a clinical failure. Third, since anti-angiogenic therapy may be chronic, normal physiological processes that require angiogenesis for homeostasis may be impaired. This not only includes the obvious angiogenic tissues, such as healing wounds and the uterine lining, but may also include a physiological response to cardiac ischaemia or peripheral vascular disease. Thus, long-term anti-angiogenic therapy may have substantial and even life-threatening side-effects. For the best clinical results, anti-angiogenic therapy should perhaps be used in combination with antineoplastic drugs [48, 49]. In any event, the central role of angiogenesis in tumour growth, progression and metastasis makes the tumour neovasculature a promising therapeutic target.

Table 1. *Current and potential clinical anti-angiogenic agents*

Angiostatin
Antibodies to specific angiogenic factors
cm 101
Endostatin
Interferons
Kinase inhibitors (suramin, genestein)
Pentosan polysulfate
Platelet factor 4
Protease inhibitors (Batimistat, Marimistat)
Receptor antagonists to specific angiogenic factors
Tetracyclins
Thalidomide
TNP-470

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