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Angiogenesis and Metastasis

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INTRODUCTION

DESPITE IMPROVEMENTS in early diagnosis, surgical techniques and local and systemic therapies, most deaths from cancer result from metastases that are resistant to conventional therapies [1–3]. Cancer metastasis consists of multiple, complex, interacting, and interdependent steps (Figure 1) [1]. Each step in the process is rate-limiting; failure to complete any one prevents the tumour cell from producing a metastasis. It is now well established that the process of angiogenesis is essential to the growth of both primary and metastatic tumours, in that growth beyond the size of 1–2 mm^3 requires tumours to develop an adequate blood supply [4]. The induction of angiogenesis is mediated by positive and negative regulatory molecules released by both tumour and host cells [5, 6]. Its biological outcome is determined by the balance of these mediators, which in the case of tumour growth favours angiogenesis. Central to the biology of tumour angiogenesis is the contribution of the host micro-environment [7, 8]. In this paradoxical process, the host contributes to the growth of tumours through the physiological response of wound healing. The body recognises tumours as wounds, but as Dvorak described, 'wounds that do not heal' [9]. In this review, we will outline the role of angiogenesis in the process of metastasis and its regulatory factors.

TUMOUR ANGIOGENESIS

Angiogenesis is mediated by multiple molecules that are released by both tumour cells and host cells including endothelial cells, epithelial cells, mesothelial cells and leucocytes. Among these molecules are members of the fibroblast growth factor (FGF) family, vascular endothelial growth factor (VEGF) (or vascular permeability factor, vasculotropin), interleukin-8 (IL-8), angiogenin, angiotropin, epidermal growth factor (EGF), fibrin, nicotinamide, platelet-derived endothelial cell growth factor (PD-ECGF), platelet-derived growth factor (PDGF), transforming growth factor-α (TGF-α), TGF-β, and tumour necrosis factor-α (TNF-α) [4, 5, 10–12]. Angiogenesis consists of sequential processes emanating from microvascular endothelial cells [4]. To generate capillary sprouts, endothelial cells must proliferate, migrate and penetrate host stroma, the direction of migration generally pointing toward the source of angiogenic molecules. The capillary sprout subsequently expands and undergoes morphogenesis to yield a capillary. Although most solid tumours are highly vascular, their vessels are not identical to normal vessels of normal tissue. There are differences in cellular composition, permeability, blood vessel stability and regulation of growth [13].

With few exceptions, benign neoplasms are sparsely vascularised and tend to grow slowly; whereas malignant neoplasms are highly vascular and fast-growing [5, 10, 11]. The increase in vasculature also increases the probability that tumour cells will enter the circulation and possibly give rise to metastasis [14]. Immunohistochemical staining of breast cancer sections with antibodies against factor VIII, a protein expressed only on the surface of endothelial cells, allowed Weidner and associates to determine the density of microvessels [15, 16]. The number of microvessels in microscopic fields selected from the most vascular areas ('hot spots') of the sections correlated directly with metastasis and inversely with survival.

Most, but not all, recent studies have concluded that increased microvessel density in the areas of most intense neovascularisation is a significant and independent prognostic indicator in early-stage breast cancer (see Gasparini, pages 2485–2493 and [15–25]). Studies of other neoplasms, such as prostate cancer, melanoma, ovarian carcinoma, gastric carcinoma and colon carcinoma, also support the conclusion that the angiogenesis index is a useful prognostic factor [26–31]. However, expectation that an angiogenesis index can identify all patients with occult metastatic disease or those with probable distant metastases may be unrealistic [5, 32]. Firstly, human tumours are heterogeneous and consist of subpopulations of cells with different biological properties [1, 8, 33–38]. Heterogeneity of angiogenic molecule expression has recently been documented in human renal carcinomas and human colon carcinomas [39, 40]. Secondly, the process of cancer metastasis is sequential and selective and consists of a series of interlinked independent steps [1, 38, 41]. To produce clinically relevant metastases, tumour cells must complete all the steps in this process. Tumour cells that can induce intense angiogenesis, but cannot survive in the circulation or proliferate in distant organs will not produce metastases (Figure 2) [1, 5, 38, 41]. Like all other steps in the metastatic cascade, angiogenesis is necessary but not sufficient for the pathogenesis of a metastasis. Thirdly, although not all large angiogenic tumours can

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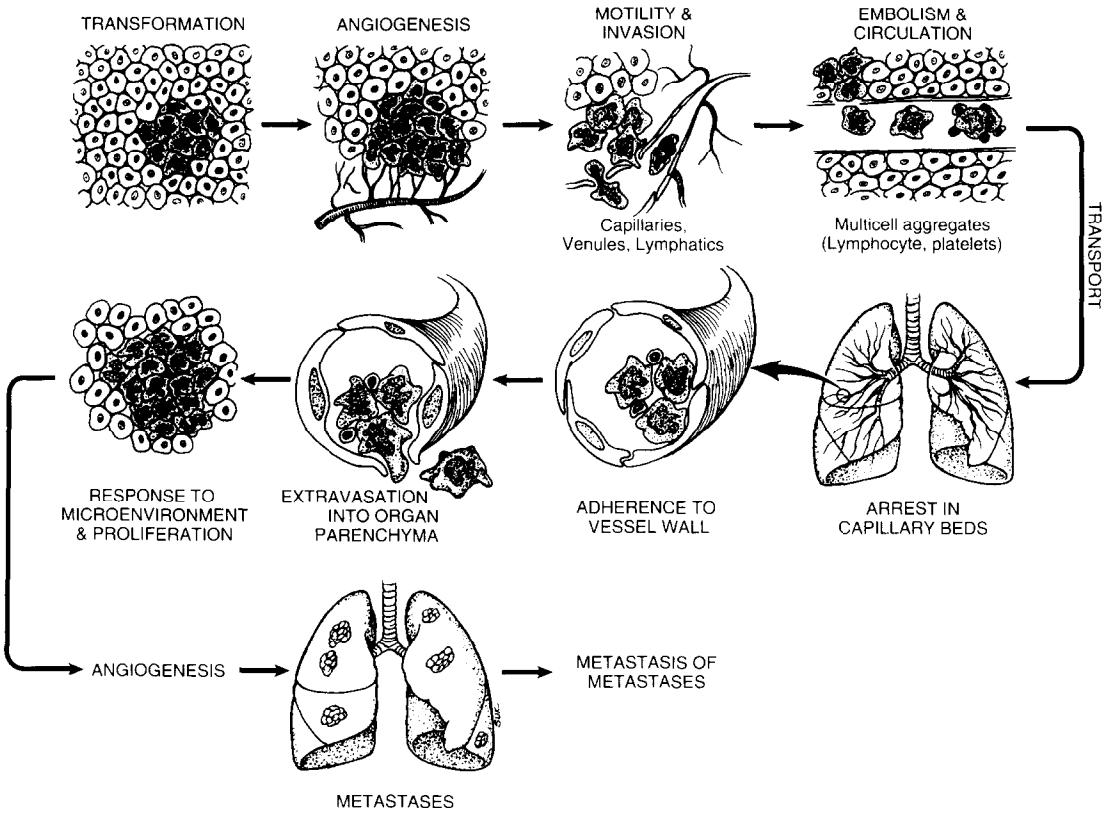

Figure 1. To produce metastases, tumour cells must detach from the primary tumour, invade the extracellular matrix and enter the circulation, survive in the circulation to arrest in the capillary bed, adhere to subendothelial basement membrane, gain entrance into the organ parenchyma, respond to paracrine growth factors, proliferate and induce angiogenesis, and evade host defences. The pathogenesis of metastasis is therefore complex and consists of multiple, sequential, selective and interdependent steps whose outcome depends on the interaction of tumour cells with homeostatic factors. Reprinted by permission of Lippincott–Raven Publishers, from Fidler IJ, Molecular biology of cancer: invasion and metastasis. In De Vita JR, Hellman S, Rosenberg SA, eds. *Cancer Principles and Practice of Oncology*, 1997, 135–152.

produce metastasis, inhibition of angiogenesis prevents the growth of tumour cells at both the primary and secondary sites and thus can prevent the development of clinically relevant metastases [10, 42].

The role of the immune system in the regulation of angiogenesis is well established. Angiogenesis is essential to homeostasis, and its extent is influenced by leucocytes, such as mast cells, T-lymphocytes and macrophages and their cytokines [7, 43–51]. Cutaneous melanoma provides a clear model of these relationships. Lymphoid-mediated angiogenesis has been recognised in cutaneous melanoma. Increased vascularity at the vertical base of human melanoma is associated with poor prognosis [52]. A local inflammatory reaction consisting of T-lymphocytes and macrophages is often associated with invasive cutaneous melanoma, and an intense inflammatory reaction is often associated with increased risk of metastasis, suggesting that inflammatory-associated angiogenesis may contribute to melanoma dissemination [53–56].

Immunological mechanisms are involved in the physiological angiogenesis that occurs subsequent to wound healing [50, 57, 58]. Systemic chemotherapy has been shown to retard wound healing, and this may be due to the decreased immune response and its contribution to wound healing; whether this is mediated by inhibition of angiogenesis is not clear [59–61]. We have investigated the role of vascularisation of tumours and its effect on tumour size in immunosuppressed mice. Similar to previous studies using immunosuppressed mice (by adult thymectomy followed by whole body X-irradiation), the subcutaneous growth of the weakly immunogenic B16 melanoma was retarded in myelosuppressed mice compared with control mice [62]. Further evidence implicating myelosuppression in the retardation of tumour growth and vascularity was obtained from doxorubicin (DXR)-pretreated animals injected with normal spleen cells one day before tumour challenge. Tumour growth in these mice was comparable with that in control mice [63]. These studies were repeated in athymic mice with very similar results, suggesting that the tumour vascularisation observed in DXR-treated mice reconstituted with spleen cells was not mediated solely by T-lymphocytes. Since reconstitution with spleen cells enhanced vascularisation of the B16 tumours, the results suggest that myelosuppressive chemotherapeutic drugs, e.g. DXR, can inhibit host-mediated vascularisation and thus inhibit tumour growth and support the concept that developing tumours can usurp homeostatic mechanisms to their advantage [8].

In a more recent study of human colon cancer specimens, we examined the role of infiltrating cells in angiogenesis [7]. Our initial studies of human colon cancer patients with various stages of disease demonstrated a correlation between VEGF expression, vessel count and metastasis formation (Figure 3) [31]. However, we identified some patients who had a high vessel count, but relatively low VEGF ex-

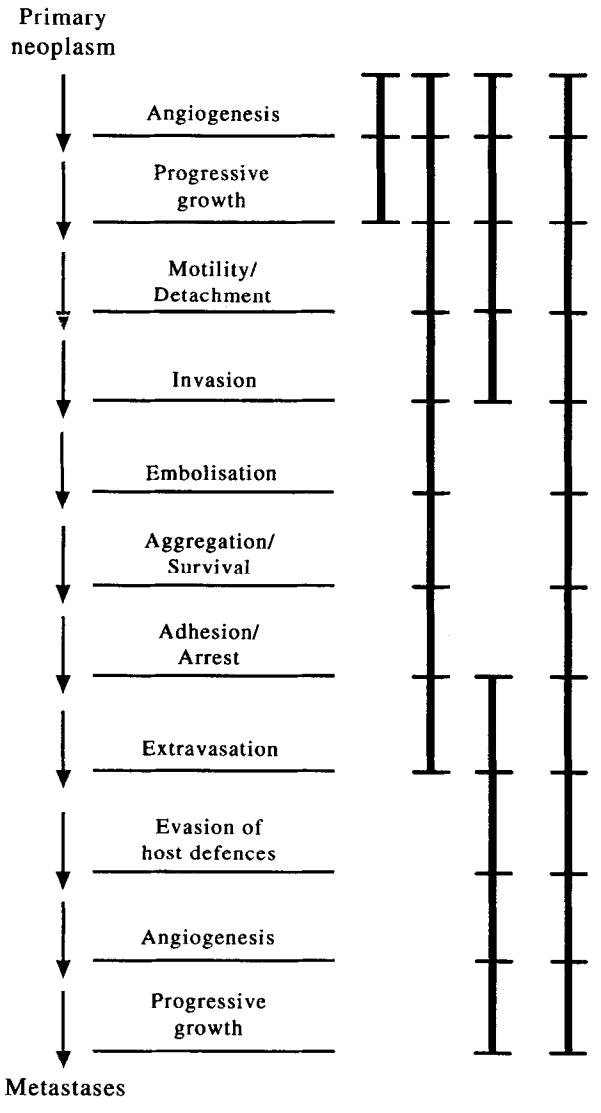

Figure 2. To produce metastases, tumour cells must complete every step in the process. If they fail to complete one or more steps, the cells are eliminated. For example, a cell that can induce angiogenesis but is not motile-invasive will not produce a metastasis. The prediction of metastatic potential, therefore, requires a multiparametric analysis. Reprinted by permission of Lippincott-Raven Publishers, from Fidler IJ, Molecular biology of cancer: invasion and metastasis. In De Vita JR, Hellman S, Rosenberg SA, eds. *Cancer Principles and Practice of Oncology*, 1997, 135–152.

pression, and so hypothesised that other factors contribute to angiogenesis. In 96 human colon cancer specimens, we found very little expression of PD-ECGF in the cancer epithelium (only 5% of patients) whereas in infiltrating cells we found that most specimens demonstrated expression of PD-ECGF in infiltrating cells (83%) [7]. Double-staining for PD-ECGF and CD68 (specific for macrophages) or CD3 (specific for lymphocytes) demonstrated many infiltrating cells simultaneously staining for PD-ECGF and CD68. Other infiltrating cells stained positive for PD-ECGF and CD3. The intensity of staining for PD-ECGF in infiltrating cells correlated with vessel counts. Northern blot analysis revealed that colon cancer specimens and normal mucosa expressed relatively high levels of PD-ECGF mRNA, whereas transcripts were not detectable in colon cancer cell lines. These data suggest that infiltrating cells may contribute to angiogenesis in human colon cancer and may provide a redundant mechanism for tumour neovascularisation [7].

REGULATION OF ANGIOGENIC AND ANTI-ANGIOGENIC FACTORS

The survival of tumours and thus their metastasis is dependent upon the balance of endogenous angiogenic and anti-angiogenic factors such that the outcome favours increased angiogenesis [5]. In many normal tissues, factors which inhibit angiogenesis predominates [10]. However, many neoplastic cells switch from an angiogenesis-inhibiting to an angiogenesis-stimulating phenotype upon transformation, as was observed in cultured fibroblasts from patients with Li-Fraumeni syndrome [64]. The switch to the angiogenic phenotype coincides with the loss of the wild-type allele of the *TP53* tumour suppressor gene, and is the result of reduced production of the anti-angiogenic factor, TSP-1 (thrombospondin 1). p53 may regulate other angiogenic molecules. In glioblastoma and hepatocellular carcinoma cell lines, mutant p53 has been shown to increase the promoter activity of the *bFGF* gene, whereas wt p53 decreased expression of bFGF [65]. Our group, as well as others, has demonstrated that mt p53 (or loss of wt p53) may increase VEGF expression [66].

Our laboratory has investigated the role of cell density in the regulation of bFGF expression in human renal cell carcinoma cells (HRCC) [67]. By *in situ* mRNA hybridisation (ISH) and Northern blot hybridisation, we found an inverse correlation between increasing cell density and bFGF expression. This finding was confirmed at the protein level as well as by immunohistochemistry and ELISA. Tumour cells harvested from dense cultures (low bFGF expression) and plated under sparse conditions expressed high levels of bFGF. Similar data were obtained in endothelial cells. The effect was not mediated by soluble factor released into the culture medium.

Recent clinical observations noting an anti-angiogenic effect of interferons (IFNs) in tumours that express high levels of bFGF led us to investigate whether IFNs could modulate the expression of bFGF [10, 68, 69]. IFNα and IFNβ but not IFNγ downregulated the expression of bFGF mRNA and protein in HRCC [70] (Figure 4). This effect was independent of the antiproliferative effects of IFNs. The downregulation of bFGF required long exposure of the cells to a low concentration of IFNs. Moreover, once IFN was withdrawn, cells resumed production of bFGF. These observations are consistent with clinical experience indicating that IFNα must be given for many months to induce a response [69]. The incubation of human bladder, prostate, colon and breast carcinoma cells with non-cytostatic concentrations of IFNα and IFNβ also inhibited bFGF production. The underlying mechanism for this modulation remains unclear.

Mechanisms regulating another angiogenic factor, VEGF, have also been investigated. Initial observations from human tumours examined by *in situ* hybridisation have demonstrated that VEGF expression is increased in necrotic areas of tumours [71, 72]. *In vitro* studies have confirmed that VEGF is increased in response to hypoxia, probably due to both increased transcription and mRNA stability [73–76].

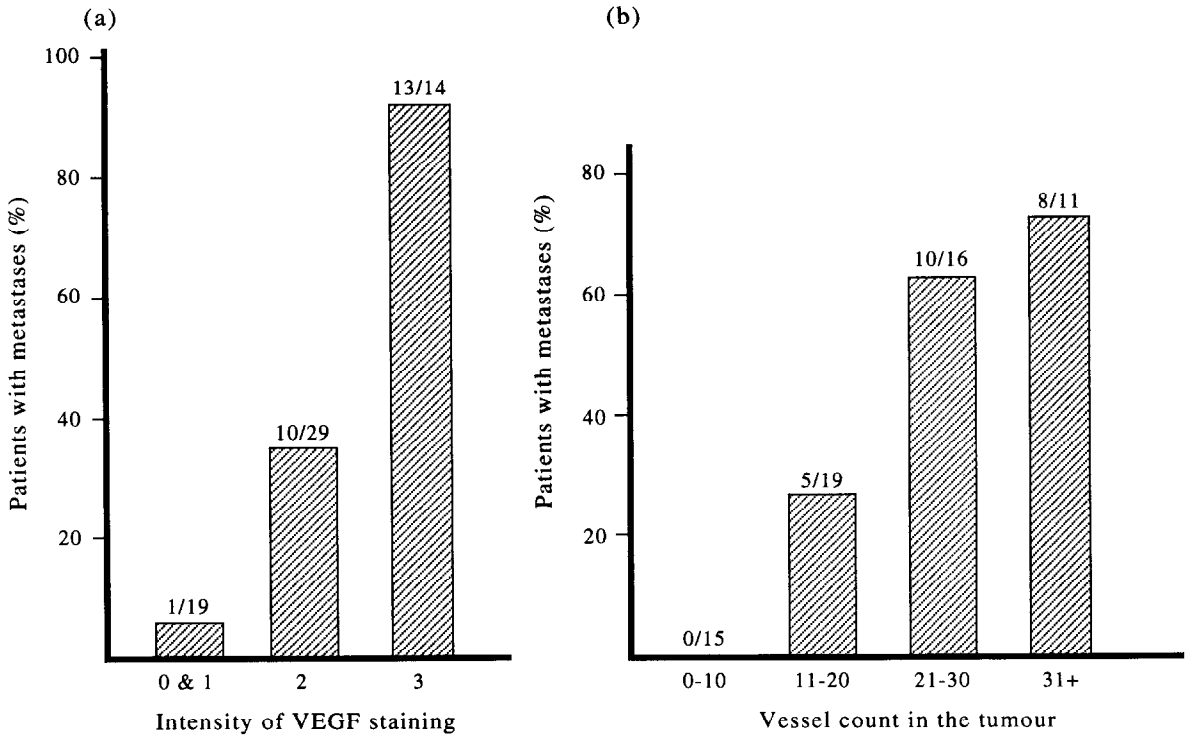

Figure 3. The relationship between VEGF expression and metastasis (a) and vessel count and metastasis (b). The prevalence of metastatic disease increases as the intensity of VEGF expression or vessel count increases. (Reproduced by permission from the American Association for Cancer Research, from Takahashi *et al.*, *Cancer Res* 1995, Vol. 55, pp. 3964–3968.

Numerous cytokines and growth factors have also been shown to increase VEGF expression. Most studies have been done in glioblastoma cell lines, a tumour system that is highly dependent upon VEGF for induction of angiogenesis. Cytokines and growth factors that have been shown to upregulate VEGF expression include IL-1, IL-6, IL-8, TGF-β, PDGF, hepatocyte growth factor and bFGF [77–84].

In contrast to the inverse correlation of cell density and bFGF expression, we found that cell density and VEGF expression were directly related [85]. Human colon carcinoma cell lines were grown under sparse and confluent conditions and VEGF mRNA expression was determined. VEGF expression was increased 2–5-fold in confluent cells compared with cells grown under sparse conditions. Cells were then plated sparsely and grown for various time periods. VEGF expression increased as cell density increased. To determine if a soluble factor mediated the increase in VEGF expression in cells grown to confluence, sparsely plated cells were grown in conditioned media from confluent cells. VEGF expression was increased in these cells, but not to the same level as cells grown to confluence.

VEGF expression is also regulated by certain oncogenes and tumour suppressor genes. *SRC* signal transduction pathways have been shown to be involved in hypoxia-mediated VEGF expression; this induction of VEGF was inhibited by genistein, an inhibitor of tyrosine kinases [86]. Studies done with v-*src* transfection into 293 and U87 cells demonstrated a 4–5-fold increase in VEGF promoter activity that corresponded to an increase in VEGF message. Overexpression of wt p53 suppressed VEGF expression in 293 cells whereas mutant p53 had no effect on VEGF [87]. Further experiments showed that transfection of wt p53 can inhibit the increased promoter activity of VEGF induced by v-*src*. This suggests that wt p53 is downstream of v-*src* [87].

In rodent and human colonic epithelial cells, transformation by activated *RAS* oncogenes upregulates VEGF [87–89]. As noted in other studies, transfection of the v-*src* oncogene also increased VEGF. That the human VEGF promoter contains four potential AP1 sites, which are key components of the *RAS* signal pathway, suggests that mutant *RAS* genes may upregulate angiogenic activity via direct transcriptional control of VEGF. Collectively, these data show that the transformation by a dominant oncogene contributes to *in vivo* tumorigenicity by both upregulation of growth factor/receptor activity and by upregulation of angiogenic molecules [64, 87–89].

Our laboratory has investigated the role of *C-SRC* in the regulation of VEGF in human colon cancer using established cell lines with decreased *C-SRC* activity (by stable antisense transfection). The tumorigenicity and growth in nude mice of these cell lines were dramatically reduced. Downregulation of *C-SRC* activity caused a 2–8-fold decrease in the cellular mRNA expression of VEGF, with the decrease proportional to the decrease in *C-SRC* activity. To determine if downregulation of *SRC* kinase activity and VEGF expression was biologically significant, conditioned medium was then added to endothelial cells in culture, and growth curves were determined. Endothelial cells grown in conditioned media from HT29 cells with decreased *C-SRC* activity exhibited a decrease in growth compared to endo-

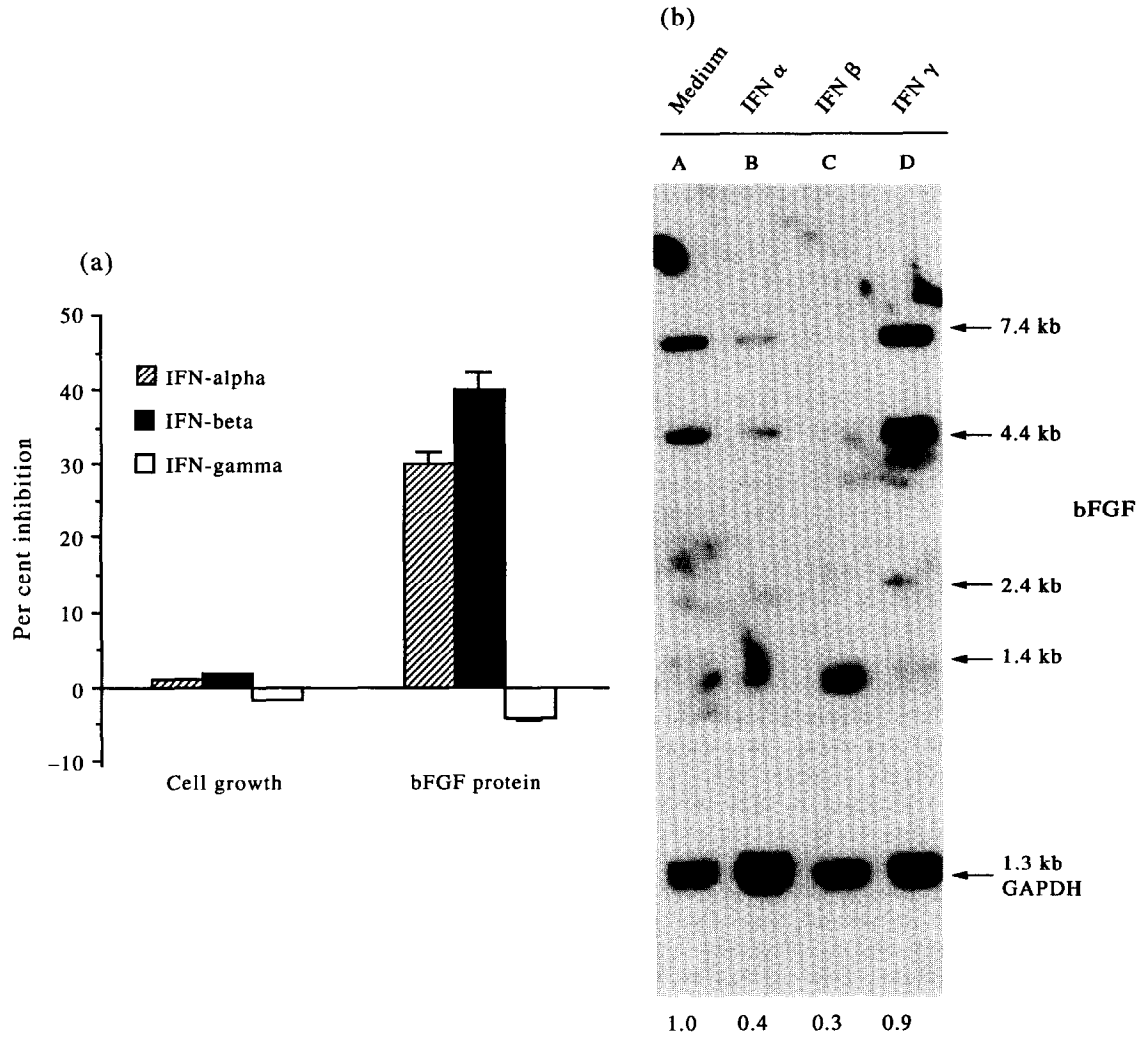

Figure 4. IFN-α and IFN-β inhibit steady-state mRNA expression and protein production of bFGF in the renal cell carcinoma cell line SN12PM6. Cells were incubated for 6 h in medium (control) or medium containing 100 U of IFN-α, IFN-β or IFN-γ per ml. (a) Per cent cytostasis and level of bFGF protein. Values are mean + S.D. of triplicate cultures. (b) Northern blot analysis. Reproduced by permission from the National Academy of Sciences, from Singh *et al., Proc Natl Acad Sci USA* 1995, Vol. 92, pp. 4562–4566.

thelial cells grown in conditioned media from the control cell lines.

ORGAN MICRO-ENVIRONMENT REGULATION OF METASTASIS/ANGIOGENESIS RELATED GENES

In 1889, Stephen Paget noted that patients with breast cancer had a disproportionate incidence of metastasis to the ovaries and that the incidence of skeletal metastasis was different for individual tumours [90]. Paget hypothesised that metastasis occurred only when favoured tumour cells (the 'seed') had a special affinity for the organ of metastasis (the 'soil'). Therefore, for tumour cells to form a metastasis successfully, the tumour cells must be able to grow in a compatible organ environment.

We recently defined three principles supporting the 'seed' and 'soil' hypothesis [8]: firstly, that neoplasms are biologically heterogeneous consisting of subpopulations of cells with different angiogenic, growth, invasive and metastatic properties; secondly, that the process of metastasis is the culmination of a series of steps including growth, invasion, embolism, adhesion and angiogenesis; and thirdly, that the outcome of metastasis is the outcome of multiple interactions between the tumour cell and host homeostatic mechanisms.

Our laboratory has demonstrated that expression of certain angiogenic factors is dependent upon the site of implantation of tumour cells. When HRCC was implanted in different organ micro-environments in nude mice, the expression of bFGF was 10 to 20 times higher in those tumours implanted in the kidney than in those implanted in the subcutaneous tissues [40]. The kidney tumours were more highly vascularised than tumours implanted in the subcutis. In sharp contrast, the expression of IFNβ was high in and around the subcutaneous tumours, whereas no IFNβ was found in the HRCC tumours growing in the kidney. This study also demonstrated that bFGF expression differed between the parental cell line and metastatic clone. The

alteration in bFGF level by the site of implantation was due to adaptation to the organ micro-environment in as much as cells re-established in culture returned to the levels found *in vitro* after 4 weeks in culture [40].

The organ-specific expression of IL-8 (an angiogenic growth factor associated with metastatic potential of melanoma cells) was examined in two human melanoma cell lines [91, 92]. The A375P (parental) and A375SM (metastatic clone) lines were implanted into the subcutis, spleen (producing liver metastasis), and tail vein (producing lung metastasis). By Northern blot and immunohistochemical analyses, subcutaneous tumours expressed the greatest amount of IL-8, followed by lung lesions, and lastly liver lesions. The expression of IL-8 in the metastatic clone was higher in tissue culture than the parental line, but relative levels of IL-8 in the other organs were similar to the parental line. This effect was not due to the size, density or subpopulation of cells. Cells cocultured with keratinocytes or well-differentiated human hepatoma cells produced similar relative amounts of IL-8 as found in *in vivo* tumour extracts. Stimulation with cytokines indigenous to those particular organs again produced similar results to the *in vivo* studies.

To determine the role of site of tumour implantation on VEGF expression, tumour angiogenesis, tumour cell proliferation and metastasis formation, we implanted a gastric cancer cell line (KKLS) in orthotopic (stomach) and ectopic (subcutaneous) locations in nude mice [93]. Tumours in the stomach demonstrated greater vascularisation, higher levels of VEGF expression, and greater proliferation than tumours in the subcutaneous tissues. In addition, 70% (7/10) of the tumours implanted in the stomach produced metastasis, but no metastases were evident in mice whose tumours were implanted subcutaneously. These data suggest that the expression of VEGF, vascularisation, metastasis and proliferation of human gastric cancer cells are regulated, in part, by the organ micro-environment.

Ongoing studies are examining the mechanism by which genes are regulated at specific sites. The possibilities include cell-to-cell contact and changes in the cytoskeleton of tumour cells regulating gene expression. Alternatively, a paracrine mechanism may be responsible for activation or inactivation of certain genes through receptor binding and signal transduction pathways. Understanding the mechanisms that regulate site-specific expression of invasion and metastasis/angiogenesis-related genes should allow more rational development of site-specific therapies for metastasis to individual organs.

MULTIPARAMETRIC *IN SITU* HYBRIDISATION STUDIES OF bFGF AND METASTASIS-RELATED GENES

Since multiple steps must be completed in order for a tumour cell to form a metastasis, we investigated the expression of several metastasis-related genes in human colon cancer specimens of various stages of disease [39]. For the study of angiogenesis, we investigated bFGF expression by both *in situ* hybridisation and Northern blot analysis. Our initial studies examined expression of bFGF in patients with node-negative tumours without evidence of distant metastasis (Dukes' stage B, Astler-Coller modification), with node-positive tumours without other evidence of metastasis (Dukes' stage C), and with distant metastasis (Dukes' stage D). By *in situ* hybridisation, the level of bFGF was significantly higher in patients with Dukes' stages C or D disease than in those with Dukes' stage B disease. Of particular interest is the fact that Northern blot hybridisation did not detect mRNA transcripts for bFGF. Careful analysis by *in situ* hybridisation revealed that bFGF was expressed at the highest levels in a subpopulation of cells at the periphery of the tumour (invasive edge), which probably represents the most 'active' portion of the invading tumour. This observation supports our postulate that tumours are heterogeneous for cells with various degrees of expression of invasion and metastasis-related genes and that a subpopulation of cells within a tumour gives rise to distant metastasis [1, 34, 40, 94]. Tumours subjected to homogenisation and analysis (i.e. Northern or Western blot) investigates gene expression of not only tumour cells, but also infiltrating cells, stromal cells and endothelial cells; i.e. the average level of gene expression in cancers with a heterogeneous cell population. The use of *in situ* hybridisation is necessary to identify a particular subpopulation of cells that may have the necessary gene expression to progress through the metastatic cascade.

In a follow-up study, we utilised the same multiparametric *in situ* hybridisation technique in an attempt to predict disease recurrence in patients with colon cancer [95]. Again, for the study of angiogenesis, we examined expression of bFGF. We found that bFGF expression was highest in patients who presented with metastatic disease. However, we were also able to identify patients who appeared to be free of metastasis at the time of initial surgery (Dukes' stage B) yet developed distant metastasis at a later date; these patients had relatively high bFGF expression along with increased expression of other metastasis related genes. Again, in these Dukes' stage B tumours we found areas of zonal heterogeneity with the highest level of bFGF expressed at the invasive edge of the tumour. Thus, identifying a subpopulation of cells with increased expression of metastasis/angiogenesis-related genes within a heterogeneous tumour may help to identify patients at risk of developing subsequent metastasis.

CONCLUSION: ANGIOGENESIS OF METASTASIS AND ITS ROLE IN CLINICAL ONCOLOGY

What role might this new knowledge of angiogenesis play in the care of the patient with cancer? A primary clinical utility of angiogenesis is the use of an angiogenesis index as a prognostic marker. In numerous solid malignancies, vessel count has been shown to be associated with metastasis formation [16, 28, 30, 96–104]. However, several recent studies have questioned this observation [25, 105, 106]. This controversy may arise from different methodologies utilised in assessing vessel counts. In addition, it is also possible that some tumours are less angiogenesis-dependent than others. For example, we have shown that in the intestinal-type gastric cancer (which typically spreads to the liver and may be several centimetres in size), vessel counts correlate with stage of disease and metastasis formation [30]. In contrast, vessel counts in diffuse-type gastric cancers (which typically spread by way of direct implantation of small peritoneal metastases and may not require a high degree of angiogen-

esis for dissemination) do not correlate with metastasis. Furthermore, vessel counts in the diffuse-type gastric cancer are lower than that in the intestinal-type gastric cancer. This may also hold true for pancreatic cancer where we have not found a correlation between vessel count and metastasis (unpublished data). In these types of cancers, the degree of neovascularisation may not be of prognostic value. Discordant results from studies examining the prognostic value of tumour vessel count may also be due to a failure to recognise that angiogenesis is but one step in the multistep process of metastasis [1, 5, 107]. If a primary tumour has a high angiogenic index and yet does not express other factors necessary for metastasis formation (i.e. adhesion/cohesion molecules, motility factors, growth factor receptors, etc). then, despite the high degree of angiogenesis, metastasis will not occur.

The recognition that tumours with a high angiogenic index may be associated with subsequent metastasis suggests that these patients may be the ones most likely to benefit from adjuvant therapy. In a recent study, evaluating the prognostic role of angiogenesis in late stage lung carcinoma, adjuvant therapy improved survival in patients with a high vessel count, but not in patients with a low vessel count [108]. However, the observation that patients with highly angiogenic tumours benefit from adjuvant therapy is not universal. In a study of node-positive breast cancer patients treated with adjuvant chemotherapy or hormonal therapy, those with a high microvessel density were found to have a worse prognosis than those with less tumour vascularity [109]. Perhaps anti-angiogenic therapy is indicated in these patients. Obviously, well-controlled clinical trials should be designed in order to determine the efficacy of anti-angiogenic therapy as an adjuvant in the treatment of patients with highly vascularised solid malignancies.

Anti-angiogenic therapy is an area of avid basic science and clinical research [69, 110–118]. As will be discussed in other sections in this Special Issue, both endogenous and man-made anti-angiogenic agents have been investigated. Since primary tumour growth is often controlled with surgery and/or irradiation, anti-angiogenic agents may be most beneficial in the treatment of widespread metastatic disease. However, several principles must be understood. Firstly, 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. Secondly, the endpoint of anti-angiogenic therapy would not be tumour shrinkage, but rather tumour stabilisation. This endpoint is new in clinical trials: tumour stabilisation over a period of time should be considered a desirable event rather than a clinical failure. Thirdly, 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 (pages 2461–2466). In any event, the central role of angiogenesis in tumour growth, progression and metastasis provides a promising therapeutic target.

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