Pergamon

European Journal of Cancer, Vol. 34, No. 2, pp. 214–221, 1998

Printed in Great Britain
0959-8049/98 $19.00+0.00

PII: S0959-8049(97)10129-0

Original Paper

Mechanisms of Tumour Metastasis

T. Meyer and I.R. Hart

Richard Dimbleby Department of Cancer Research/ICRF Laboratory, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, U.K.

INTRODUCTION

THE ABILITY of malignant tumours to metastasise largely is responsible for their lethality. Such is the intransigent nature of this process that patients with metastatic disease from a solid tumour almost invariably are incurable. Thus, despite the advent of better local treatment in the form of surgery and radiotherapy and systemic chemotherapy, the clinical challenge in oncology remains that of combating metastatic spread. There is, therefore, a pressing need to understand the underlying molecular and cellular mechanisms of tumour dissemination so as to develop novel therapies based on this knowledge. This review cannot be exhaustive and instead will focus on several selected areas of investigation which may, eventually, hold potential for intervention.

THE METASTATIC CASCADE

The metastatic cascade is illustrated schematically in Figure 1. Having disengaged from its primary site the metastatic tumour cell must invade the surrounding stroma, enter the vasculature or lymphatic system, survive and arrest at a distant site. From here it extravasates into the tissue and, after elaboration of a blood supply, grows and develops into a secondary mass. The parallels between many of these steps and those involved in the migratory behaviour of leucocytes during inflammation or the initiation of new tissue growth and angiogenesis during wound healing have provided the identity of many of the molecules thought to be involved in tumour dissemination. Key processes involved include changes in cellular adhesion, the production of proteolytic enzymes capable of degrading the stroma and the secretion of a variety of cytokines which attract and activate stromal cells and endothelial cells during invasion and angiogenesis.

CELL ADHESION MOLECULES (CAMs)

The initial escape of a tumour cell from its primary site requires the loss of cell–cell attachment which, in epithelial tumours, is mediated largely by the members of the cadherin family and in particular by E-cadherin. Alterations also occur in the nature of adhesion events between the released tumour cells and the extracellular matrix which allow the motile neoplastic cells to migrate over underlying substrates. Integrins are of prime importance in these cell-substrate interactions. Other adhesion molecules, such as those of the immunoglobulin superfamily and/or the selectin family, are involved in several heterophilic cell–cell interactions including the adherence of tumour cells to the endothelium. There are then many steps in tumour cell spread which involve cell–cell and cell-substratum adhesion receptors and which may depend upon changes in the functional status of these molecules.

CADHERINS

Cadherins are a family of calcium-dependent cell adhesion molecules which mediate predominantly homotypic cell–cell interactions and play a key role during morphogenesis as well as in maintenance of the differentiated phenotype. They are trans-membrane glycoproteins with a highly conserved cytoplasmic tail which interacts with the cytoskeletion via the intracellular proteins α, β and γ catenins. Deletion of the cytoplasmic tail, or modification of the catenin, abrogates cadherin function suggesting that the catenins have a major role in regulating adhesive activity. The cadherin family contains several members, including E-cadherin which is involved in epithelial cell–cell adhesion.

Following the demonstration that differentiated kidney epithelial cells (MDCK) adopted a fibroblastic morphology and became invasive in collagen gels in response to monoclonal antibodies (MAb) against E-cadherin [1, 2], additional *in vitro* evidence of the importance of this molecule in controlling cell invasion was soon acquired. Loss of E-cadherin in breast cancer cell lines was correlated with the presence of other markers of the metastatic phenotype such as vimentin, *in vitro* invasiveness and a fibroblastoid phenotype [3]. In an oesophageal cell line, E-cadherin negative clones lacked intracellular adhesion and had increased invasiveness [4], whilst the *de novo* expression of E-cadherin in the breast line MDA MB 231 reduced the ability of tumour cells to form osteolytic bone metastases in nude mice [5]. Interestingly, in the breast cancer cell subline MCF7/6 tamoxifen was found to restore E-cadherin expression and to suppress the invasive phenotype [6, 7]; an unexpected effect which might partially explain some of the antimetastatic effects of this drug.

These experimental results have been paralleled by an ever-growing number of immunohistochemical studies documenting loss or reduction of E-cadherin expression in

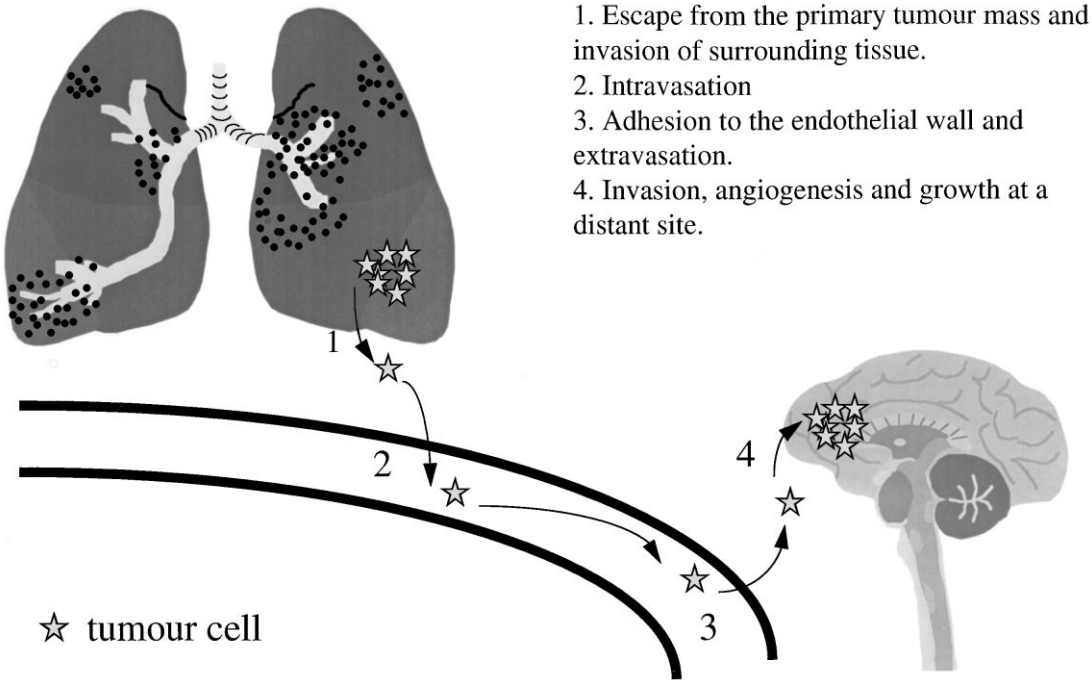

Figure 1. Stages of metastasis.

malignant disease. Consistent loss of E-cadherin expression has been reported in lobular breast cancer [8–10], although expression is also reduced in poorly differentiated ductal carcinoma *in situ* [11] and invasive ductal carcinoma [9]; in the latter, such reduction has been found to correlate with other poor prognostic indicators such as grade [9]. Similar correlations between loss of E-cadherin protein and increasing malignancy have been demonstrated in carcinoma of the prostate [12–14], stomach [15], bladder [16–18], colorectum [19, 20] and pancreas [21].

However, loss of E-cadherin protein is not the only way that cell–cell cohesion could be abrogated. Changes in catenin expression could also lead to loss of cadherin function. In squamous cell carcinoma of the oesophagus, reduction of alpha catenin correlates with poor prognosis [22] and, in a small series, reduced alpha catenin was found to be associated with metastasising breast carcinomas [23]. The findings that the adenomatous polyposis coli (APC) gene product, which is mutated in many sporadic and inherited tumours, associates with α and β catenin was the first indication that the catenins might have a wider role in malignancy than simply through modulating cellular adhesion [24]. In a recent series of reports [25–28], it has become clear that β-catenin also binds to the DNA binding proteins of the T cell factor-lymphoid enhancer factor (Tcf-Lef) family. Normally the APC protein, in concert with glycogen synthase kinase (GSK)-3β, binds and regulates levels of free β-catenin by regulating degradation. In APC mutant cells, the levels of free β-catenin rise and, by binding to Tcf-Lef, drive gene expression. It has been shown that some of the genes expressed as a consequence of these events may be involved in inhibiting apoptosis or in stimulating cellular proliferation. Since mutations in β-catenin also can activate this β-catenin-Tcf signalling pathway [27] it is clear that this protein also can act as an 'oncogene'.

Thus, the role of the cadherin-catenin complex in regulating tumour progression is not attributable solely to effects on cell adhesion and migration [29], but probably also encompasses modification of neoplastic cell growth and survival.

INTEGRINS

Integrins comprise a diverse family of receptors that mediate adhesion between the cell membrane and the extracellular matrix (ECM) or other cell adhesion molecules (CAMs). They are composed of two non-covalently associated α and β subunits and the combination of 8 β subunits with at least 14 α subunits generates an heterogeneous family of over 20 heterodimers. Ligand specificity is determined largely by the subunit composition and whilst some receptors are promiscuous and bind a wide range of ligands, others are more specific. It was initially thought that integrins functioned merely as transmembrane rivets linking the cells to the ECM, but in recent years it has become evident that integrins can transduce signals [30] and influence functions as diverse as migration, differentiation and apoptosis [31–33]. Furthermore, the cell is able to activate or inactivate certain integrins in response to other signals, thus allowing rapid modulation of adhesive and de-adhesive activity. Evidence that integrins are involved in metastasis comes from both histological and *in vitro* studies.

Differences in integrin expression between normal and malignant tissue have been documented for many tissue types. In epithelial tumours, there is a considerable amount of heterogeneity, but some consistent patterns have emerged. A reduction or change in the distribution of laminin and collagen receptors has been found in breast [34, 35], pancreas [36, 37], colon [38–41] and lung [42, 43] cancers suggesting that loss of attachment to the basement membrane, which is composed largely of laminin and type IV collagen, is important for facilitating tumour development. Intriguingly, Natali [44] has observed normal levels of expression of the laminin receptor, α6β4, in breast cancer metastases in the lymph node compared with that observed in the primary tumour or

pleural metastases where levels were reduced. This raises the possibility of this occurring by clonal selection (i.e. the transit to or the arrest of specific subsets of tumour cells by different organs) or modulation of integrin expression by the host tissue micro-environment. Not all tumour types manifest a generalised down-regulation of integrin expression. Thus, in contrast to epithelial tumours, melanoma has been shown to exhibit an up regulation of integrins, including αvβ3, during the vertical growth phase of tumour growth and in the metastases [45].

There is much experimental data supporting the probable role of integrins in modulating metastasis. Several studies have examined the effect of over-expressing integrins in recipient tumour cells. Transfection of transformed Chinese hamster ovary cells with the fibronectin receptor, α5β1, reduced their migration, their proliferation rate and rendered them non-tumorigenic [46]. Similarly, re-expression of α2β1 in a poorly differentiated mammary carcinoma caused the cells to revert to a more differentiated phenotype and to exhibit loss of tumorigenicity [47]. Although these data fit well with the naturally occurring observed changes in human disease, other investigators have shown that the adhesive interactions mediated by integrins are a necessary requirement for metastasis formation. Thus, the co-injection of a peptide, containing the recognition site of many integrins, the so-called RGD binding motif, with B16-F10 murine melanoma cells inhibited the formation of experimental lung metastases in recipient syngeneic mice [48]. Both subcutaneous tumour growth and experimental lung metastasis, resulting from the implantation of the human M21 melanoma line into nude mice, were inhibited by 17E6, a MAb directed against the αv subunit [49]. Similarly, with a human breast cancer cell line, experimental metastasis was inhibited by MAbs against the α5β1 fibronectin receptor [50], whilst inoculation of nude mice with a polymeric form of fibronectin inhibited the subsequent formation of metastasis by melanoma, osteosarcoma and colon carcinoma cell lines [51].

There is an apparent paradox here, with integrins being down-regulated in the more progressed stages of many human tumours and yet apparently being important molecules for facilitating the occurrence of metastasis. It recently has been shown that integrin-dependent migration is affected by three variables, namely substratum ligand density, level of integrin expression and integrin-ligand binding affinity [52]. These different parameters may have a profound influence upon the behaviour of different tumour types. Clearly the nature of the substrate will vary according to anatomical location and both integrin expression and integrin affinity can be modulated by local cytokines [53, 54]. Thus, although immunohistochemical studies imply that changes in integrin expression are important, they cannot tell us anything about the functional activity of such molecules. It may be that a receptor is highly expressed but has a low level of activation or vice versa. We have observed this phenomenon in breast cancer cell lines which express the vitronectin receptors αvβ1 and αvβ5. Those lines derived from tumours adhered well to vitronectin via these integrins yet the cell line MCF10A, derived from benign breast disease, adhered poorly to the same substrate despite exhibiting equivalent levels of cell surface expression of these molecules [55]. Another study utilising breast cancer cell lines has shown that the level of α2β1 integrin expression is not clearly related to any particular phenotype, but mediates binding to collagen by all expressing cells. However, this receptor was found to bind to laminin only in the more differentiated lines that were shown to be E-cadherin and oestrogen receptor positive [56]. Clearly the simple immunohistochemical measurement of receptor levels alone must be interpreted with caution in the absence of much of this additional information.

THE IMMUNOGLOBULIN SUPERFAMILY

A number of cell adhesion molecules have been shown to contain one or more Ig-like domains and thus are classified as members of the immunoglobulin superfamily. These cell surface receptors are involved in both homophilic and heterophilic interactions with a variety of different ligands and play major roles in determining embryonic and neural development, wound healing and inflammation.

It has been proposed that these molecules are involved specifically in the process of leucocyte interaction with, and extravasation across, the endothelial wall. During inflammation, cytokines including TNFα and interleukin-1, induce the expression of such immunoglobulin family members as ICAM-1, ICAM-2, V-CAM and PECAM on the endothelial wall. These interact with a number of integrins expressed on the surface of leucocytes causing them to arrest prior to extravasation. This physiological process has provided a paradigm for the way in which haematogenous metastasis might occur. ICAM-1 and ICAM-2 bind to the β2 integrins expressed on circulating leucocytes [57] and, since ICAM-1 has also been shown to be a marker of progression in malignant melanoma [58], it is conceivable that an indirect interaction with leucocytes may mediate binding between the tumour cell and the endothelium, thus allowing its enhanced extravasation at a metastatic site. Equally the integrin α4β1, which binds to VCAM [59], may subserve a similar role in renal cell tumours, melanoma and sarcomas where α4β1 has been found to be upregulated in more advanced disease [60–62]. PECAM has also recently been shown to bind αvβ3 [63], an integrin which, as already stated, appears to be expressed at an enhanced level at the cell surface in advanced malignant melanoma. Direct evidence for the role of this family of adhesion receptors in the metastatic process is, however, scant and most of the attributed activities are based upon speculation.

SELECTINS

Members of the selectin family were identified initially on platelets, leucocytes and endothelium (P, E and L selectin, respectively). The calcium-dependent lectin domain situated at the distal end of the extracellular portion of the molecule binds to the carbohydrate ligand sialyl-Le^x which is expressed on neutrophils and monocytes. During inflammation, selectin expression is induced and is then responsible for the low affinity binding which initiates leucocyte rolling, which is followed by arrest and extravasation mediated by the integrin, Ig superfamily interaction. Again, this process may serve as a paradigm for the mechanisms involved in tumour cell arrest and extravasation.

Sialyl Le^x and its isoform sialy-Le^a have been found on several different carcinomas (colon [64] and stomach [65]) and such cells have been shown to bind to activated endothelium [66] by this mechanism. In colorectal cancer, the increased expression of sialy-Le^x has been shown to correlate with clinical stage and metastatic potential [67] and, in a study of selectin ligands in skin tumours, metastatic

squamous cell carcinomas were all found to express sialyl-Le^x whereas it was absent from normal skin and basal cell carcinoma [68].

OTHER CELL ADHESION MOLECULES

Several other CAMs, including CD44 and the 67–69 kDa laminin-elastin binding protein, have been implicated as having a role in metastatic spread, but examination of their involvement in this process is beyond the scope of this review and the reader is referred to other sources for further discussion [69, 70].

THE ROLE OF PROTEOLYTIC ENZYMES

An essential step in the metastatic cascade is breach of the basement membrane and invasion of the surrounding stroma. There are a wide range of proteolytic enzymes that might contribute to this process. Many of these have been shown to have prognostic implications when identified in tumour samples or to increase the invasiveness of cells in an experimental setting.

There are three main families of proteolytic enzymes thought to be involved in tumour malignancy: (1) the serine proteases which include urokinase plasminogen activator (uPA), elastase, plasmin and cathepsin G; (2) the matrix metalloproteinases which comprise the gelatinases, the interstitial collagenases, the stromelysins and matrilysin; and (3) the cysteine proteases, the cathepsins B and L.

The regulation of enzyme activity is complex and is a function not only of the level of expression of the enzyme itself, but also of the concentration of activating and inhibitory factors; the production and secretion of any of which could be affected during malignant transformation.

UROKINASE PLASMINOGEN ACTIVATOR AND ITS RECEPTOR

The uPA/uPAR system has been shown to play a key role in many physiological processes including embryogenesis, angiogenesis and wound healing and there now is considerable evidence for its importance in a wide spectrum of tumour types.

uPA is secreted in an autocrine or paracrine manner as an inactive single chain precursor pro-uPA which binds to a glycosyl-phosphatidyl-inositol (GPI) linked membrane receptor (uPAR) [71]. On binding it is cleaved to an active two chain enzyme by membrane bound plasmin and, in this form, catalyses the conversion of plasminogen to plasmin.

Plasmin in turn can directly degrade circulating or tissue proteins as well as activate zymogens, mainly of the matrix metalloproteinase family, or growth factors. The activity of uPA also is regulated by four inhibitors, plasminogen activator inhibitor (PAI)-1, 2 and 3 and protease nexin 1. PAI 1 is associated with the extracellular matrix protein vitronectin which acts as a carrier molecule and forms a complex with the receptor-bound uPA. The complex is then internalised, partially degraded and the uPAR re-expressed on the cell surface. The conversion of receptor bound pro-uPA to its active form proceeds at a rate 20-fold higher when bound to its receptor rather than when in solution and the system thus provides a means of initiating a cascade of proteolysis which can be focused at a particular point on the cell surface. *In vitro*, it has been noted in several cell types that uPAR localises to focal contacts where it is associated with integrins [72, 73]. Furthermore, the uPAR is able to serve as a cell surface receptor and mediate adhesion to vitronectin [74] and influence integrin function [75]. In summary, the uPA/UPAR system provides a means of directing proteolysis at sites of focal contacts and could well be a pivotal feature of neoplastic migration.

Immunohistochemical studies have revealed components of this proteolytic system to be located at the invasive edge of cancers and also in the associated stromal cells [76], suggesting that complex interactions exist between tumour and stroma during the invasive process. In several tumour types, uPA, uPAR and PAI 1 have emerged as prognostic indicators. In breast cancer, for example, higher tumour levels of uPA have been associated with higher relapse rate [77] and, in univariate analysis, found to be a better discriminator for disease-free survival than lymph node status, tumour size or oestrogen receptor levels [78]. High uPAR levels in breast cancers have also been linked to a worse prognosis [79] and, in one study, the expression level in the stroma was found to correlate more positively with relapse than did expression in the neoplastic cells themselves [80]. Similar observations have also been made in colorectal cancer [81] and gastric cancer [82].

Experimental data also suggest a role for the uPA/uPAR system in modulating the aggressive behaviour of tumours and this knowledge offers some prospects for future therapeutic intervention. In a rat breast cancer model, over-expression of uPAR increased the *in vitro* invasiveness of such cells and also led to the growth of larger tumours and the enhanced occurrence of metastasis [83]. Moreover, uPA over-expression in human prostate cells led to earlier spinal cord compression and more widespread skeletal metastases when such transfected cells were injected into the left cardiac ventricle of nude rats [84]. Conversely, MAbs against uPA, when injected into mice inoculated with syngeneic tumour cells, significantly inhibited the development of lung metastasis [85]. Recently antisense technology has also been used to down-regulate uPAR expression in human glioblastoma and human squamous cell carcinoma cell lines resulting in decreased *in vitro* invasiveness of treated cells relative to their control counterparts [86, 87].

MATRIX METALLOPROTEINASES

The matrix metalloproteinases (MMPs) are a diverse family of zinc-dependent endopeptidases with a broad spectrum of activity. They can be divided according to their substrate specificity into the gelatinases, the collagenases and the stromelysins and they are secreted as inert zymogens which become activated by cleavage of the amino terminus in the extracellular milieu. *In vitro* their expression can be increased by a variety of growth factors and cytokines and their conversion to active enzymes can be induced by plasmin or trypsin. Equally their activity can be inhibited by three known tissue inhibitors of MMPs (termed TIMP 1, 2 and 3) whose own expression also is influenced by local cytokines and growth factors. Thus, during normal physiological events the activity of these enzymes is tightly regulated. In malignant disease this regulation can be disrupted, contributing to the increased invasion and tissue destruction which is a hallmark of malignant disease.

The best evidence for the importance of these enzymes in the metastatic process comes from work on the type IV collagenases or gelatinases (MMP-2 and MMP-9) whose expression is increased in several tumour types compared

with normal adjacent tissue [88]. The metastatic potential of many neoplastic cells can be blocked by the introduction of cDNAs encoding for the TIMPS [89]. Expression of the degradative enzymes is not, however, restricted solely to the tumour and, in colorectal cancer for example, high expression of MMP-2 and TIMP-2 has been documented in the adjacent stromal cells [90].

As mentioned above, the activity of the MMPs and TIMPs can be influenced, often differentially, by growth factors such as TGF-β and bFGF which are released from the ECM during degradation. This phenomenon may serve to amplify the degradative process and also emphasises the dynamic nature of the metastatic cascade whilst illustrating the importance of the micro-environment.

Stromelysin 3 initially was identified specifically in the stromal cells associated with breast carcinoma tissue [91] and subsequently has been found in a variety of other tumour types, including squamous cell carcinoma of the head and neck [92] and colon cancer [93]. Recently, we have shown that reporter genes placed under the transcriptional control of the Stromelysin 3 promoter can be upregulated by conditioned medium from some breast carcinoma cell lines. These results suggest that the tumour releases cytokines which are capable of stimulating the stromal cells to upregulate proteases and thereby participate in matrix degradation [94].

The possibility of blocking matrix degradation as a therapeutic strategy is currently being explored in phase 2 clinical trials. The MMP inhibitor, Batimastat, has been shown to inhibit human tumour growth and spread in nude mice [95] and its orally administered derivative, Marimastat, is undergoing clinical evaluation.

ANGIOGENESIS

It is now well established that for a tumour to grow beyond 1–2 mm in diameter, an independent blood supply is required [96] and this neovascularisation is itself an important prerequisite for the release of tumour cells into the circulation [97].

The impact of angiogenesis in clinical terms has been demonstrated in many tumour types, including breast and lung, in which microvessel density has been shown to correlate with metastasis and survival [98].

Both tumour cells and stromal cells, including the endothelial cells themselves, each produce a number of positive and negative regulators of angiogenesis which act in either a paracrine or autocrine manner to control endothelial cell growth. It is the fine balance between these various factors in the local environment which seems to be crucial in determining the eventual angiogenic response.

There is an ever growing list of so-called angiogenic factors (VEGF, bFGF, FGF, PDGF, ILGF 1 and TNFα) although the validity of this specific label is, in many cases, unconfirmed by experiments of appropriate rigour. One angiogenic factor which is not subject to such caveats is vascular endothelial growth factor (VEGF) [100]. The action of this growth factor appears to be restricted to endothelial cells and its production is stimulated by hypoxia, as occurs frequently in tumours, making it likely to play a major role in determining neovascularisation *in vivo*. Indeed the inhibition of this growth factor using antibodies has been shown to slow tumour growth in selected experimental models [101].

The presence of angiogenic factors alone may not be sufficient to induce new vessel formation in that such stimulating agents have to function in the presence of inhibitory factors. One such angiogenesis-inhibitory factor is thrombospondin which is produced by normal cells, but often is down-regulated in tumorigenesis [102]. Interestingly thrombospondin expression appears to be regulated by p53 and fibroblasts from patients with Li-Fraumeni syndrome, which have lost functional p53 and have low thrombospondin levels, can be rendered non-tumorigenic by the re-introduction of cDNA encoding for p53 which allows re-expression of thrombospondin [103]. Another inhibitor, angiostatin, recently has been identified in the murine Lewis lung carcinoma tumour model [104]. This protein, which is present in the circulation of tumour-bearing animals but disappears when the primary tumour is removed, appears to suppress the induction of angiogenesis. Removal of the primary tumour led to a decline in levels of angiostatin and a concomitant increase in metastasis development [104]. Thus, local production of VEGF, which has a half life of three minutes and very low levels in the circulation, has been suggested to be sufficient to maintain angiogenesis in the primary tumour but angiostatin, which has a half life of 2.5 days, suppresses angiogenesis in metastatic tumours [104]. The precise significance of this in human tumorigenesis is unclear at the present time, but it not only serves as an example of the complex interactions between various factors in the maintenance and growth of metastases, but may also help to explain the apparent dormancy of metastasis on the one hand and, on the other hand, the metastatic burst sometimes observed following primary tumour clearance [105]. A number of other inhibitors of angiogenesis also have been identified, including α- and β-interferon, Interleukin-8 and endostatin [106], which gives rise to the hope that such naturally occurring substances may hold potential for therapy.

As a therapeutic target, tumour angiogenesis has several attractions. It is envisaged that because only proliferating endothelium is targeted, toxicity would be low. Additionally the problem of drug resistance is overcome because endothelial cells lack the inherent genetic instability which facilitates the emergence of drug-resistant clones in populations of transformed cells. Tumour penetration would not be so important for any anti-angiogenic drugs which would naturally reach the endothelial cells lining the blood vessel lumena, while any such approach might be used in combination with conventional cytotoxic chemotherapy [107].

Several compounds already have shown promise in *in vivo* models, including fumagillin [108] and platelet factor-4 [109], and it is likely that many more such drugs will be developed in the next few years. It is worth noting, in this context, that inhibitors of proteolytic enzymes, designed to prevent tumour cell-mediated destruction and invasion, also will block endothelial-cell derived proteases involved in the invasion of these blood vessel cells during the neovascularisation process. The search for antigens specific to proliferating endothelial cells has identified the vitronectin receptor as a possible target [110]. Antibodies directed against αvβ3 have been shown to inhibit cytokine-induced angiogenesis in the chick chorioallantoic membrane model [111] and the growth of breast cancer in a human chimera model in the SCID mouse [112]. Recently, subcutaneous injections of a cyclic peptide containing the RGD sequence have successfully inhibited retinal neovascularisation induced by hypoxia [113]. These encouraging preliminary results suggest that the

targeting of the tumour-associated angiogenic response may provide a means of regulating cancer growth and metastatic development.

CONCLUSIONS

Improved understanding of the molecular basis of metastatic spread promises to identify novel targets for therapy. Already agents developed from such knowledge have found their way into the clinic and this situation is likely to accelerate in the near future with the identification of novel gene products involved in regulating tumour malignancy.

1. Behrens J, Mareel M, Van Roy FM, Birchmeier W. Dissecting tumor cell invasion: epithelial cells acquire invasive properties after the loss of uvomorulin-mediated cell–cell adhesion. *J Cell Biol* 1989, **108**, 2435–2447.
2. Behrens J. The role of cell adhesion molecules in cancer invasion and metastasis. *Breast Cancer Res Treat* 1993, **24**, 175–184.
3. Sommers CL, Thompson EW, Torri JA, Kemler R, Gelmann EP, Byers SW. Cell adhesion molecule uvomorulin expression in human breast cancer cell lines: relationship to morphology and invasive capacities. *Cell Growth and Differ* 1991, **2**, 365–372.
4. Doki Y, Shiozaki H, Tahara H, *et al.* Correlation between E-cadherin expression and invasiveness *in vitro* in a human esophageal cancer cell line. *Cancer Res* 1993, **53**, 3421–3426.
5. Mbalaviele G, Dunstan CR, Sasaki A, Williams PJ, Mundy GR, Yoneda T. E-cadherin expression in human breast cancer cells suppresses the development of osteolytic bone metastases in an experimental metastasis model. *Cancer Res* 1996, **56**, 4063–4070.
6. Bracke ME, Charlier C, Bruyneel EA, Labit C, Mareel MM, Castronovo V. Tamoxifen restores the E-cadherin function in human breast cancer MCF-7/6 cells and suppresses their invasive phenotype. *Cancer Res* 1994, **54**, 4607–4609.
7. Charlier C, Bruyneel E, Lechanteur C, Bracke M, Mareel M, Castronovo V. Enhancement of tamoxifen-induced E-cadherin function by Ca2+ channel antagonists in human breast cancer MCF7/6 cells. *Eur J Pharmacol* 1996, **317**, 413–416.
8. Gamallo C, Palacios J, Suarez A, *et al.* Correlation of E-cadherin expression with differentiation grade and histological type in breast carcinoma. *Am J Pathol* 1993, **142**, 987–993.
9. Palacios J, Benito N, Pizarro A, *et al.* Anomalous expression of P-cadherin in breast carcinoma. Correlation with E-cadherin expression and pathological features. *Am J Pathol* 1995, **146**, 605–612.
10. Siitonen SM, Kononen JT, Helin HJ, Rantala IS, Holli KA, Isola JJ. Reduced E-cadherin expression is associated with invasiveness and unfavorable prognosis in breast cancer. *Am J Clin Pathol* 1996, **105**, 394–402.
11. Gupta SK, Douglas Jones AG, Jasani B, Morgan JM, Pignatelli M, Mansel RE. E-cadherin (E-cad) expression in ductal carcinoma *in situ* (DCIS) of the breast. *Virchows Arch* 1997, **430**, 23–28.
12. Umbas R, Schalken JA, Aalders TW, *et al.* Expression of the cellular adhesion molecule E-cadherin is reduced or absent in high-grade prostate cancer. *Cancer Res* 1992, **52**, 5104–5109.
13. Umbas R, Isaacs WB, Bringuier PP, *et al.* Decreased E-cadherin expression is associated with poor prognosis in patients with prostate cancer. *Cancer Res* 1994, **54**, 3929–3933.
14. Cheng L, Nagabhushan M, Pretlow TP, Amini SB, Pretlow TG. Expression of E-cadherin in primary and metastatic prostate cancer. *Am J Pathol* 1996, **148**, 1375–1380.
15. Matsui S, Shiozaki H, Inoue M, *et al.* Immunohistochemical evaluation of alpha-catenin expression in human gastric cancer. *Virchows Arch* 1994, **424**, 375–381.
16. Bringuier PP, Umbas R, Schaafsma HE, Karthaus HF, Debruyne FM, Schalken JA. Decreased E-cadherin immunoreactivity correlates with poor survival in patients with bladder tumours. *Cancer Res* 1993, **53**, 3241–3245.
17. Shimazui T, Schalken JA, Giroldi LA, *et al.* Prognostic value of cadherin-associated molecules (alpha-, beta-, and gamma-catenins and p120cas) in bladder tumors. *Cancer Res* 1996, **56**, 4154–4158.
18. Syrigos KN, Krausz T, Waxman J, *et al.* E-cadherin expression in bladder cancer using formalin-fixed, paraffin-embedded tissue: correlation with histopathological grade, tumour stage and survival. *Int J Cancer* 1995, **64**, 367–370.
19. Dorudi S, Sheffield JP, Poulsom R, Northover JM, Hart IR. E-cadherin expresion in colorectal cancer. An immunocytochemical and *in situ* hybridization study. *Am J Pathol* 1993, **142**, 981–986.
20. Dorudi S, Hanby AM, Poulsom R, Northover J, Hart IR. Level of expression of E-cadherin mRNA in colorectal cancer correlates with clinical outcome. *Br J Cancer* 1995, **71**, 614–616.
21. Pignatelli M, Ansari TW, Gunter P, *et al.* Loss of membranous E-cadherin expression in pancreatic cancer: correlation with lymph node metastasis, high grade, and advanced stage. *J Pathol* 1994, **174**, 243–248.
22. Nakanishi Y, Ochiai A, Akimoto S, *et al.* Expression of E-cadherin, alpha-catenin, beta-catenin and plakoglobin in esophageal carcinomas and its prognostic significance: immunohistochemical analysis of 96 lesions. *Oncology* 1997, **54**, 158–165.
23. Rimm DL, Sinard JH, Morrow JS. Reduced alpha-catenin and E-cadherin expression in breast cancer [see comments]. *Lab Invest* 1995, **72**, 506–512.
24. Su LK, Vogelstein B, Kinzler KW. Association of the APC tumor suppressor protein with catenins. *Science* 1993, **262**, 1734–1737.
25. Behrens J, von Kries JP, Kuhl M, *et al.* Function interaction of β-caterin with the transcription factor LEF-1. *Nature* 1996, **382**, 638–642.
26. Korinek V, Barker N, Morin PJ, *et al.* Constitutive transcriptional activation by a β-catenin-Tcf complex in $APC^{-/-}$ colon carcinoma. *Science* 1997, **275**, 1784–1787.
27. Morin PJ, Sparks AB, Korinek V, *et al.* Activation of β-catenin-Tcf signaling in colon cancer by mutations in β-catenin or APC. *Science* 1997, **275**, 1787–1790.
28. Rubinfeld R, Robbins P, El-Gamil M, Albert I, Porfiri E, Polakis P. Stabilization of β-catenin by genetic defects in melanoma cell lines. *Science* 1997, **275**, 1790–1792.
29. Chen H, Paradies NE, Fedor-Chaiken M, Brackenbury R. E-cadherin mediates adhesion and suppresses cell motility via distinct mechanisms. *J Cell Sci* 1997, **110**, 345–356.
30. Hynes RO. Integrins: versatility, modulation, and cell adhesion. *Cell* 1992, **69**, 11–25.
31. Brooks PC, Montgomery AM, Rosenfeld M, *et al.* Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 1994, **79**, 1157–1164.
32. Montgomery AM, Reisfeld RA, Cheresh DA. Integrin alpha v beta 3 rescues melanoma cells from apoptosis in three-dimensional dermal collagen. *Proc Natl Acad Sci USA* 1994, **91**, 8856–8860.
33. Cheresh DA, Mecham RP. Integrins: Molecular and Biological Responses to the Extracellular Matrix (Monograph). London Academic Press Inc., 1994.
34. Gui GPH, Wells CA, Yeomans P, Jordan SE, Vinson GP, Carpenter R. Integrin expression in breast cancer cytology—a novel predictor of axillary metastasis. *Eur J Surg Oncol* 1996, **22**, 254–258.
35. Koukoulis GK, Virtanen I, Korhonen M, Laitinen L, Quaranta V, Gould VE. Immunohistochemical localization of integrins in the normal, hyperplastic, and neoplastic breast. Correlations with their functions as receptors and cell adhesion molecules. *Am J Pathol* 1991, **139**, 787–799.
36. Weinel RJ, Rosendahl A, Pinschmidt E, Kisker O, Simon B, Santoso S. The alpha 6-integrin receptor in pancreatic carcinoma. *Gastroenterology* 1995, **108**, 523–532.
37. Hall PA, Coates P, Lemoine NR, Horton MA. Characterization of integrin chains in normal and neoplastic human pancreas. *J Pathol* 1991, **165**, 33–41.
38. Pignatelli M, Smith ME, Bodmer WF. Low expression of collagen receptors in moderate and poorly differentiated colorectal adenocarcinomas. *Br J Cancer* 1990, **61**, 636–638.
39. Stallmach A, von Lampe B, Matthes H, Bornhoft G, Riecken EO. Diminished expression of integrin adhesion molecules on human colonic epithelial cells during the benign to malignant tumour transformation. *Gut* 1992, **33**, 342–346.
40. Koretz K, Schlag P, Boumsell L, Moller P. Expression of VLA-alpha 2, VLA-alpha 6 and VLA-beta 1 chains in normal

mucosa and adenomas of the colon, and in colon carcinomas and their liver metastases. *Am J Pathol* 1991, **138**, 741–750.
41. Lindmark G, Gerdin B, Pahlman L, Glimelius B, Gehlsen K, Rubin K. Interconnection of integrins alpha 2 and alpha 3 and structure of the basal membrane in colorectal cancer: relation to survival. *Eur J Surg Oncol* 1993, **19**, 50–60.
42. Suzuki S, Takahashi T, Nakamura S, *et al.* Alterations of integrin expression in human lung cancer. *Japanese J Cancer Res* 1993, **84**, 16–74.
43. Roussel E, Gingras MC, Ro JY, Branch C, Roth JA. Loss of alpha 1 beta 1 and reduced expression of other beta 1 integrins and CAM in lung adenocarcinoma compared with pneumocytes. *J Surg Oncol* 1994, **56**, 198–208.
44. Natali PG, Nicotra MR, Botti C, Mottolese M, Bigotti A, Segatto O. Changes in expression of alpha 6 beta 4 integrin heterodimer in primary and metastatic breast cancer. *Br J Cancer* 1992, **66**, 318–322.
45. Albelda SM, Mette SA, Elder DE, *et al.* Integrin distribution in malignant melanoma: association of the β3 subunit with tumour progression. *Cancer Res* 1990, **50**, 6757–6764.
46. Giancotti FG, Ruoslahti E. Elevated levels of the alpha 5 beta 1 fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. *Cell* 1990, **60**, 849–859.
47. Zutter MM, Santoro SA, Staatz WD, Tsung YL. Re-expression of the alpha 2 beta 1 integrin abrogates the malignant phenotype of breast carcinoma cells. *Proc Natl Acad Sci USA* 1995, **92**, 7411–7415.
48. Humphries MJ, Olden K, Yamada KM. A synthetic peptide from fibronectin inhibits experimental metastasis of murine melanoma cells. *Science* 1986, **233**, 467–470.
49. Mitjans F, Sander D, Adan J, *et al.* An anti-alpha v integrin antibody that blocks integrin function inhibits the development of a human melanoma in nude mice. *J Cell Sci* 1995, **108**, 2825–2838.
50. Newton SA, Reeves EJ, Gralnick H, *et al.* Inhibition of experimental metastasis of human breast carcinoma in athymic nude mice by anti-α5β1 fibronectin receptor integrin antibodies. *Int J Oncology* 1995, **6**, 1063–1070.
51. Pasqualini R, Bourdoulous S, Koivunen E, Woods V, Rouslahti E. A polymeric form of fibronectin has antimetastatic effects against multiple tumour types. *Nature Medicine* 1996, **2**, 1197–1203.
52. Palecek SP, Loftus JC, Ginsberg MH, Lauffenburger DA, Horwitz AF. Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. *Nature* 1997, **385**, 537–540.
53. Herzberg F, Schoning M, Schirner M, Topp M, Thiel E, Kreuser ED. IL-4 and TNF-alpha induce changes in integrin expresion and adhesive properties and decrease the lung-colonizing potential of HT-29 colon-carcinoma cells. *Clin Exp Metastasis* 1996, **14**, 165–175.
54. Kim LT, Yamada KM. The regulation of expression of integrin receptors. *Proc Soc Exp Biol Med* 1997, **214**, 123–131.
55. Meyer T, Marshall JF, Hart IR. Expression of alpha v integrins and vitronectin receptor identity in breast cancer cell lines. *Br J Cancer* 1997, in press.
56. Maemura M, Akiyama SK, Woods VLJr, Dickson RB. Expression and ligand binding of alpha 2 beta 1 integrin on breast carcinoma cells. *Clin Exp Metastasis* 1995, **13**, 223–235.
57. Springer TA. Adhesion receptors of the immune system. *Nature* 1990, **346**, 425–434.
58. Natali P, Nicotra MR, Cavaliere R, *et al.* Differential expression of intercellular adhesion molecule 1 in primary and metastatic melanoma lesions. *Cancer Res* 1990, **50**, 1271–1278.
59. Elices MJ, Osborn L, Takada Y, *et al.* VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell* 1990, **60**, 577–584.
60. Tomita Y, Saito T, Saito K, Oite T, Shimizu F, Sato S. Possible significance of VLA-4 (alpha 4 beta 1) for hematogenous metastasis of renal-cell cancer. *Int J Cancer* 1995, **60**, 753–758.
61. Hart IR, Birch M, Marshall JF. Cell adhesion receptor expression during melanoma progression and metastasis. *Cancer Metastasis Rev* 1991, **10**, 115–128.
62. Paavonen T, Tiisala S, Majuri ML, Bohling T, Renkonen R. *In vivo* evidence of the role of alpha 4 beta 1-VCAM-1 interaction in sarcoma but not in carcinoma extravasation. *Int J Cancer* 1994, **58**, 298–302.
63. Buckley CD, Doyonnas R, Newton JP, *et al.* Identification of alpha v beta 3 as a heterotypic ligand for CD31/PECAM-1. *J Cell Sci* 1996, **109**, 437–445.
64. Dejana E, Martin Padura I, Lauri D, *et al.* Endothelial leukocyte adhesion molecule-1-dependent adhesion of colon carcinoma cells to vascular endothelium is inhibited by an antibody to Lewis fucosylated type I carbohydrate chain. *Lab Invest* 1992, **66**, 324–330.
65. Sakamoto S, Watanabe T, Tokumaru T, Takagi H, Nakazato H, Lloyd KO. Expression of Lewis^a, Lewis^b, Lewis^x, Lewis^y, siayl-Lewis A, and sialyl-Lewis^x blood group antigens in human gastric carcinoma and in normal gastric tissue. *Cancer Res* 1989, **49**, 745–752.
66. Takada A, Ohmori K, Takahashi N, *et al.* Adhesion of human cancer cells to vascular endothelium mediated by a carbohydrate antigen, sialyl Lewis A. *Biochem Biophys Res Commun* 1991, **179**, 713–719.
67. Nakamori S, Kameyama M, Imaoka S, *et al.* Increased expression of sialyl Lewis^x antigen correlates with poor survival in patients with colorectal carcinoma: clinicopathological and immunohistochemical study. *Cancer Res* 1993, **53**, 3632–3637.
68. Groves RW, Allen MH, Ross EL, Ahsan G, Barker JN, MacDonald DM. Expression of selectin ligands by cutaneous squamous cell carcinoma. *Am J Pathol* 1993, **143**, 1220–1225.
69. Albelda SM. Role of integrins and other cell adhesion molecules in tumor progression and metastasis. *Lab Invest* 1993, **68**, 4–17.
70. Hart I, Hogg N. Cell adhesion and cancer. Monograph. New York, Cold Spring Harbor Laboratory Press, 1995.
71. Blasi F. Urokinase and Urokinase receptor: a paracrine/autocrine system regulating cell migration and invasiveness. *BioEssays* 1993, **15**, 105–111.
72. Pollanen J, Hedman K, Nielsen LS, Dano K, Vaheri A. Ultrastructural localization of plasma membrane-associated urokinase-type plasminogen activator at focal contacts. *J Cell Biol* 1988, **106**, 87–95.
73. Xue W, Mizukami I, Todd RF, Petty RH. Urokinase-type plasminogen activator associates with β1 and β3 integrins of fibrosarcoma cells: dependence on extracellular matrix components. *Cancer Res* 1997, **57**, 1682–1689.
74. Wei Y, Waltz DA, Roa N, Drummond RJ, Rosenberg S, Chapman HA. Identification of the urokinase receptor as an adhesion receptor for vitronectin. *J Biol Chem* 1994, **269**, 32380–32388.
75. Wei Y, Lukashev D, Simon DI, *et al.* Regulation of integrin function by the urokinase receptor. *Science* 1996, **273**, 1551–1555.
76. Pyke C, Kristensen P, Ralfkiaer E, *et al.* Urokinase-type plasminogen activator is expressed in stromal cells and its receptor in cancer cells at invasive foci in human colon adenocarcinomas. *Am J Pathol* 1991, **138**, 1059–1067.
77. Janicke F, Schmitt M, Hafter R, *et al.* Urokinase-type plasminogen activator (u-PA) antigen is a predictor of early relapse in breast cancer. *Fibrinolysis* 1990, **4**, 69–78.
78. Duffy MJ, Reilly D, O'Sullivan C, O'Higgins N, Fennelly JJ, Andreasen P. Urokinase-plasminogen activator, a new and independent prognostic marker in breast cancer. *Cancer Res* 1990, **50**, 6827–6829.
79. Duggan C, Maguire T, McDermott E, O'Higgins N, Fennelly JJ, Duffy MJ. Urokinase plasminogen activator and urokinase plasminogen activator receptor in breast cancer. *Int J Cancer* 1995, **61**, 597–600.
80. Kim SJ, Shiba E, Taguchi, T, *et al.* Urokinase type plasminogen activator receptor is a novel prognostic factor in breast cancer. *Anticancer Research* 1997, **17**, 1373–1378.
81. Ganesh S, Sier CF, Griffioen G, *et al.* Prognostic relevance of plasminogen activators and their inhibitors in colorectal cancer. *Cancer Res* 1994, **54**, 4065–4071.
82. Nekarda H, Schmitt M, Ulm K, *et al.* Prognostic impact of urokinase-type plasminogen activator and its inhibitor PAI-1 in completely resected gastric cancer. *Cancer Res* 1994, **54**, 2900–2907.

83. Xing RH, Rabbani SA. Overexpression of urokinase receptor in breast-cancer cells results in increased tumor invasion, growth and metastasis. *Int J Cancer* 1996, **67**, 423–429.
84. Achbarou A, Kaiser S, Tremblay G, *et al.* Urokinase overproduction results in increased skeletal metastasis by prostate cancer cells *in vivo*. *Cancer Res* 1994, **54**, 2372–2377.
85. Hearing VJ, Law LW, Corti A, Appella E, Blasi F. Modulation of metastatic potential by cell surface urokinase of murine melanoma cells. *Cancer Res* 1988, **48**, 1270–1278.
86. Mohanam S, Chintala SK, Go Y, *et al. In vitro* inhibition of human glioblastoma cell line invasiveness by antisense uPA receptor. *Oncogene* 1997, **14**, 1351–1359.
87. Kook YH, Adamski J, Zelent A, Ossowski L. The effect of antisense inhibition of urokinase receptor in human squamous cell carcinoma on malignancy. *Embo J* 1994, **13**, 3983–3991.
88. Stetler Stevenson WG. Type IV collagenases in tumor invasion and metastasis. *Cancer Metastasis Rev* 1990, **9**, 289–303.
89. Montgomery AM, Mueller BM, Reisfeld RA, Taylor SM, DeClerck YA. Effect of tissue inhibitor of the matrix metalloproteinases-2 expression on the growth and spontaneous metastasis of a human melanoma cell line. *Cancer Res* 1994, **54**, 5467–5473.
90. Poulsom R, Pignatelli M, Stetler Stevenson WG, *et al.* Stromal expression of 72kda type IV collagenase (MMPa-2) and TIMP-2 mRNAs in colorectal neoplasia. *Am J Pathol* 1992, **141**, 389–396.
91. Basset P, Bellocq JP, Wolf C, *et al.* A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. *Nature* 1990, **348**, 699–704.
92. Muler D, Wolf C, Abecassis J, *et al.* Increased stromelysin 3 gene expression is associated with increased local invasiveness in head and neck squamous cell carcinomas. *Cancer Res* 1993, **53**, 165–169.
93. Newell KJ, Witty JP, Rodgers WH, Matrisian LM. Expression and localization of matrix-degrading metalloproteinases during colorectal tumorigenesis. *Mol Carcinog* 1994, **10**, 199–206.
94. Ahmad A, Marshall JF, Basset P, Anglard P, Hart IR. Modulation of human stromelysin three promoter and gene expression by human breast cancer. *Int J Cancer* 1997, **13**(2), 290–296.
95. Wang X, Fu X, Brown PD, Crimmin MJ, Hoffman RM. Matrix metalloproteinase inhibitor BB-94 (batimastat) inhibits human colon tumour growth and spread in a patient-like orthotopic model in nude mice. *Cancer Res* 1994, **54**, 4726–4728.
96. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 1990, **82**, 4–6.
97. Liotta LA, Kleinerman J, Saidel GM. Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation. *Cancer Res* 1974, **34**, 997–1004.
98. Craft PS, Harris AL. Clinical prognostic significance of tumour angiogenesis. *Ann Oncol* 1994, **5**, 305–311.
99. Risau W. What, if anything, is an angiogenic factor? *Cancer Metastasis Rev* 1996, **15**, 149–151.
100. Claffey KP, Robinson GS. Regulation of VEGF/VPF expression in tumor cells: consequences for tumor growth and metastasis. *Cancer Metastasis Rev* 1996, **15**, 165–176.
101. Kim KJ, Li B, Winer J, *et al.* Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth *in vivo*. *Nature* 1993, **362**, 841–844.
102. Rastinejad F, Polverini PJ, Bouck NP. Regulation of the activity of a new inhibitor of angiogenesis by a cancer suppressor gene. *Cell* 1989, **56**, 345–355.
103. Dameron KM, Volpert OV, Tainsky MA, Bouck N. Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science* 1994, **265**, 1582–1584.
104. O'Reilly MS, Holmgren L, Shing Y, *et al.* Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma [see comments]. *Cell* 1994, **79**, 315–328.
105. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1995, **1**, 27–31.
106. O'Reilly MS, Boehm T, Shing Y, *et al.* Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 1997, **88**, 277–285.
107. Teicher BA. A systems approach to cancer therapy. (Antioncogenics+standard cytotoxics → mechanism(s) of interaction). *Cancer Metastasis Rev* 1996, **15**, 247–272.
108. Ingber D, Fujita T, Kishimoto S, *et al.* Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. *Nature* 1990, **348**, 555–557.
109. Maione TE, Gray GS, Petro J, *et al.* Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. *Science* 1990, **247**, 77–79.
110. Pasqualini R, Koivunen E, Rouslahti E. αv integrins as receptors for tumor targeting by circulating ligands. *Nat Biotechnology* 1997, **15**, 542–546.
111. Brooks PB, Clark AF, Ceresh DA. Requirement of vascular integrin αvβ3 for angiogenesis. *Science* 1994, **264**, 569–571.
112. Brooks PC, Stromblad S, Klemke R, Visscher D, Sarker FH, Cheresh D. Anti integrin αvβ3 blocks human breast cancer growth and angiogenesis in human skin. *J Clin Invest* 1995, **96**, 1815–1822.
113. Hammes HP, Brownlee M, Jonczyk A, Sutter A, Preissner KT. Subcutaneous injection of a cyclic peptide antagonist of vitronectin receptor-type integrins inhibits retinal neovascularization. *Nat Med* 1996, **2**, 529–533.