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Editorial

Overview — burgeoning promise in metastasis research

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1. Introduction

This special issue of the *European Journal of Cancer* on Invasion and Metastasis comprises a wide spectrum of excellent articles by acknowledged experts and there is a clear incongruity in the Editorial being written by a scientist who has never worked in the field. However, the broad scope of these articles and the importance of the issues they address mean that this special issue deserves to appeal to a wider readership than those who are already well informed. Thus, although I approach the task with diffidence, there may be some value in the topics being introduced by someone whose perspective is from the outside, rather than from one of the specific issues addressed here.

My contact with this subject has mainly been through the need to make strategic decisions about the allocation of scarce resources to different areas of cancer research. In this context, the crucial importance of disseminated disease to clinical studies has always been acknowledged. Invasion and metastasis are the hallmarks of malignancy, the major causes of death in cancer patients and the main impediments to improving cures. However, investigations into the biology of metastasis have, until recently, compared unfavourably with the elegant and informative reductionist studies on neoplastic cell growth that have so revolutionised our understanding of cancer over the past 30 years. The reasons are obvious; metastasis requires changes in cell behaviour as well as in growth but behaviour is harder to quantify than growth and is also less amenable to analysis in lower eukaryotes. These problems are greatly magnified by the appreciation that the metastatic process is not just dependent on inherent features of the tumour cells but is also influenced by stromal and endothelial cells and by the mechanical and physiological nature of the tumour cell's environment. This complexity confounds reductionist approaches to understanding metastasis but, paradoxically, it also offers novel possibilities for inhibiting metastasis that, in recent years, have made the field one of hope rather than frustration. These issues have all been reviewed recently, for example, [1–3].

2. The metastatic process

For any kind of reductionist analysis a complex process must be broken down to its component parts and there seems to be general agreement on the sequential steps required for a primary tumour to seed a distant secondary. Most contributors to this volume have outlined this process, which is shown schematically in seven stages in Fig. 1.

The first stage is growth of the primary tumour at a localised site. Cell multiplication, allied to the genetic instability of tumour cells, favours the selection of variants able to complete some or all of the steps required for metastasis [4]. The primary can not, however, grow beyond the size of 1–2 mm without acquiring a blood supply and this could limit the scope for selected variation. Endothelial growth and migration into the tumour are, however, stimulated by hypoxia (see Dachs and Tozer, pp. 1649–1660) which induces production of factors such as the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) by the tumour and some infiltrating cells. Angiogenesis is also assisted by extracellular matrix (ECM) breakdown and it seems that matrix metalloproteases (MMPs) are expressed early in tumour growth. Indeed, Sternlicht and colleagues [5] have convincing evidence that the

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MMP, stromelysin-1, can act as a tumour promoter at the earliest stages of carcinogenesis. The tumour neovasculature is more permeable than normal vessels, possessing a fenestrated basement membrane and fewer cell junctions, which is thought to increase the risk of metastasis. However, its cells also multiply far more rapidly than normal capillary endothelium, making it particularly susceptible to some anti-angiogenic therapeutic approaches (see below and Deplanque and Harris, pp. 1713–1724).

The next two stages, of breaking through the tumour basement membrane and entering a blood or lymphatic vessel (with or without significant transit of the interstitial stroma), are effectively one process, but they can theoretically be distinguished. Both require degradation of the ECM, a network of collagens, fibronectin and glycosaminoglycans which, together with laminins, comprises the basement membrane of tissues and blood vessels and, with the alternative addition of elastins, makes up the interstitial stroma. The ECM provides cell attachment, ligands for cellular receptors (integrins, which serve support and intracellular signalling functions) and it protects and sequesters inactive forms of growth factors and proteases.

Of the proteases, MMPs (interstitial collagenases, stromelysins and gelatinases) are produced by stromal and endothelial cells, in response to tumour-produced factors, but they can also be produced by tumours themselves. They are regulated in balance with tissue inhibitors of metalloproteases (TIMPS) and their association with early tumour growth and angiogenesis has already been mentioned. A second group comprises the serine proteases; tissue-type plasminogen activator and urokinase-type plasminogen activator (u-PA), the latter being the more important in ECM degradation. The cellular receptor for u-PA (u-PAR) concentrates u-PA at, for example, cell-ECM adhesions where it may affect integrin signalling and hence link and potentially coordinate, proteolysis and cell migration (see below). Like MMPs, PAs are regulated by natural inhibitors (PAI). Further details of these activities are given in the articles by Curran and Murray (pp. 1621–1630) and Reijerkerk and colleagues (pp. 1695–1705). A third degradative activity, heparanase, which is an endoglycosidase for heparan sulphate (HS) in glycosaminoglycans, regulates HS-sequestered b-FGF and PAs [6]. As these activities would suggest, the proteases and heparanase together not only degrade ECM and activate one another but also cleave bound pro-forms of cytokines and growth factors to induce proliferation and migration of endothelial, mesenchymal and tumour cells.

Cellular movement is the defining characteristic of the invasion stage. It may be a passive translocation in response to mechanical pressure or an active migration. The latter requires, in addition to local ECM degradation, changes in cell adhesive properties and cellular locomotion (crawling). In many epithelial tumours, the adherens junction component, E-cadherin, is lost or

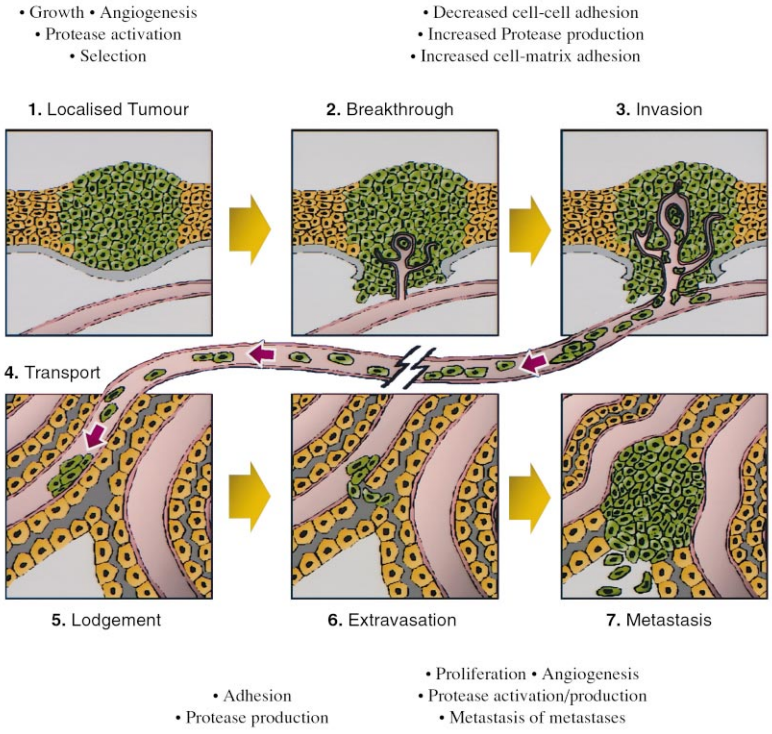

Fig. 1. The stages of the metastatic process, illustrated here for spread of a primary tumour from a surface epithelium to the liver. The arbitrary division into seven steps is reflected in the text.

reduced, leading to decreased cell–cell adhesion, thus enabling tumour cells to detach from their neighbours. As reviewed in the article by Beavon (pp. 1607–1620), E-cadherin reduction (which may be as a consequence of hypoxia) can also enhance, by complex signalling, cellular proliferation and survival and it is perhaps not surprising that low E-cadherin levels are strong markers of poor prognosis (see Heiman and Hellman, pp. 1631–1639). Reduction in cell–cell adhesion must be accompanied by maintained or enhanced cell–ECM adhesion (including integrin binding to RGD motifs in ECM proteins) to provide growth and survival signals (reviewed by Jones and colleagues, pp. 1595–1606). One consequence of integrin signalling may be an elevation of free β-catenin and hence reduced cell–cell adhesion (Beavon), so there may be a co-ordinated inverse relationship between cell adhesion to the ECM and to other cells.

The mechanical anchorage of cells to the ECM permits cell crawling by the formation and loss of ECM contacts. Jones and colleagues deal with some mechanisms for this process which occurs in response to motility factors that, together with their receptors, are often upregulated in tumours. As the review by Condeelis and colleagues (pp. 1671–1680) points out, the formation and loss of cell–ECM contacts must be polarised around blood vessels if they are to be effective in intravasation.

The next stage in metastasis is transport of tumour cells in the blood or lymphatics (see Condeelis and colleagues) to distant sites. Dogma has it that few lone tumour cells survive this hazard being destroyed by either: mechanical trauma; immune attack by natural killer cells or cytotoxic T lymphocytes (CTL); or anoikis, a programmed cell death due to lack of attachment to ECM and hence loss of survival signalling through integrins. Tumour cells can avoid destruction by clumping with one another, or with immune cells and platelets, thus reducing trauma, possibly preventing anoikis and enhancing the next stage of metastasis, lodgement. The article by Condeelis and colleagues finds that transport in the circulation (unlike intravasation or growth at a new site) is not rate limiting in metastasis, so survival may be simply stochastic. On the other hand, Jones and colleagues point out changes inherent to tumour cells that could protect against anoikis and tumours are known to avoid CTL recognition by downregulating MHC Class I or co-stimulatory molecules, implying a selective advantage for cells with effective survival strategies.

Lodgement and extravasation, the next two stages of metastasis, are not random, but the biological bases of the classical 'seed and soil' hypothesis are not clear. Anatomical and haemodynamic factors play a role, as may selective chemotaxis to soluble or fixed attractants. There is also likely to be selective cell adhesion; exposed endothelial basement membrane would be expected to offer better attachment and there could be more avid interactions between tumour cell integrins and fibronectins and other ECM molecules at the favoured sites of lodgement. Extravasation displays the same requirements as intravasation, but most tumours thereafter apoptose or remain dormant and fail to complete the final stage of metastasis, growth at the new site (see Condeelis and colleagues). This failure may be due to the lack of appropriate stimulatory growth factors and ECM or it may reflect the prevalence of inhibitory interactions. A particular case of the latter is represented by long-lived anti-angiogenic factors, such as angiostatin and endostatin that are produced by the primary tumour [2]. Their mode of action is uncertain but it is clear that, in a dormant metastasis, cell proliferation will be balanced by cell death until an angiogenic 'switch' allows proliferation to predominate [7].

3. The metastatic cell

The subdivision of the metastatic process into a number of stages and the application of animal and tissue culture assays to mimic a cell's transit of one or more of these stages (Fig. 2) offered the prospect of identifying and validating oncogenes and tumour

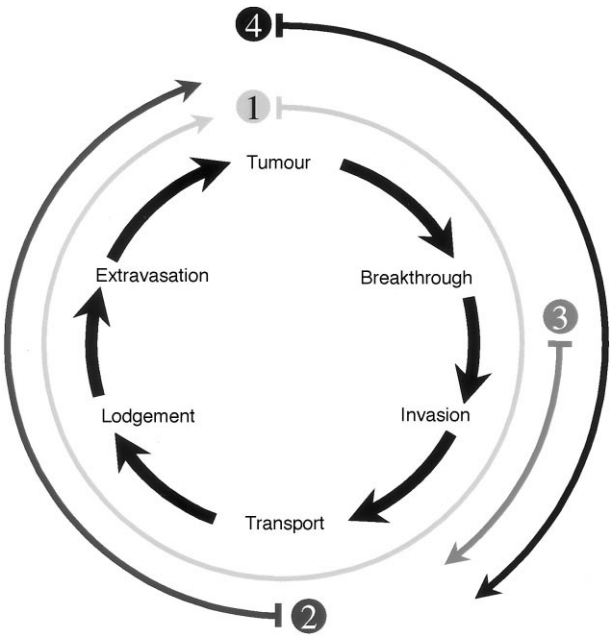

Fig. 2. Animal and tissue culture models for the metastatic process. The seven stages of metastasis are shown as a circular process, in which the primary tumour and its metastasis (stages 1 and 7) are at the top of the circle. The extents to which different experimental procedures model the process are depicted by arrows 1–4. *In vitro* invasion assays 3 include various gel migration techniques while *in vivo* invasion assays 4 are exemplified by the chick chorioallantoic membrane technique. 1, Subcutaneous/orthotopic implantation; 2, tail vein inoculation; 3, *in vitro* invasion/migration assays; 4, *in vivo* invasion assays.

suppressor genes with a specific role in metastasis. The concept that metastasis has a genetic basis, at least in some experimental models, was demonstrated by the isolation of stable tumour cell variants with enhanced metastatic capacity [4]; examples of such cells are studied in the article by Condeelis and colleagues). Cells from metastases were found to show differences in gene expression (or in post-translational modifications of gene products) from their less metastatic parents and genetic differences between primary tumours and metastases have been observed in human cancers (examples from colorectal cancer are reviewed by McLeod and colleagues, pp. 1706–1712). These observations have prompted many workers to identify genes differentially expressed in metastasis (including novel genes) in the hope of revealing the genetic specification of the metastatic phenotype or its component stages and the article by Ozanne (pp. 1640–1648) exemplifies this approach. Others have chosen to study known genes, whose functions make them candidates for roles in metastasis and the reviews by Beavon; Curran and Murray; and Dachs and Tozer provide examples. This latter approach has implicated some known growth-promoting oncogenes in various stages of metastasis although, since cell multiplication is also an integral part of metastasis, it is important to show that they affect stages of the process that do not just involve tumour growth (see Jones and colleagues).

The catalogue of functions that are genetically altered in tumour cells and that specifically affect metastasis is, so far, rather small. However, as the article by Ozanne proposes, stages of metastasis may be regulated by changes in master genes that control programmes of gene activity. In the work reviewed by Ozanne, the regulator is the transcription factor AP-1 which may itself be regulated by hypoxia (Dachs and Tozer) and adhesion linked kinases (Jones and colleagues). Downstream consequences of the activity of AP-1 need not be obligatory features of the tumour cell phenotype as it journeys through the metastatic process but they may, none the less, be pathognomic of metastasis. The notion of a programme of gene activity controlling aspects of metastasis might mean that no one function in this programme can convert a non-metastatic cell to metastasis, but each function is essential to maintain the metastatic phenotype, if only at one particular stage of the process.

The concept of the metastatic cell that carries genetic or physiological stigmata of its status is of practical importance. We do not know whether individual patients show predisposition to disseminated disease so the chief prognostic variables are the tumour cells themselves and the host response to them. As discussed by Heimann and Hellman, certain characteristics of tumour biology are prognostically useful but the prognostic value, if any, of later changes in the metastatic process has yet to be discovered. A great deal still needs to be learned about metastasis before a biological profile of a tumour will reliably predict its metastatic potential and hence be informative for treatment. For some prognostic purposes, however, such knowledge is not a prerequisite. In the critique of PCR-based methods to detect circulating tumour cells (Ghossein and Bhattacharya, pp. 1681–1694), a major requirement is to discriminate tumour from other circulating cells and, for this purpose, genes typifying the tissue of tumour origin are probably more valuable than those denoting metastatic capacity.

4. Cellular interactions in metastasis: new prospects for therapy

The metastatic cell and its descendants require certain attributes if they are to complete the process of metastasis, but it is also clear that the inherent properties of the tumour cells are not the only determinants of success in this process. Interactions with other tumour cells, stromal mesenchymal cells, endothelial and immune cells and their products can all play a part in helping or hindering tumour dissemination. As has been alluded to above, these interactions are very complex, differ at different stages of the metastatic process and are probably finely balanced between promoting and preventing metastatic progression. The review by Curran and Murray gives an example of this balance in the contradictory properties of MMPs which break down the ECM to promote angiogenesis but also cleave plasminogen and collagen XVIII to yield, respectively, the angiogenic inhibitors angiostatin and endostatin. Some of these complex interactions are shown pictorially in Fig. 3 but, as testified by many contributions to this volume, the full network of potential positive and negative influences on metastasis is too intricate to be reduced to a diagram.

A major consequence of this complexity has been the plethora of targets it offers for novel rational therapies based on pharmaceutical or genetic approaches (Dachs and Tozer; Curran and Murray; Reijerkerk and colleagues; McLeod and colleagues; Deplanque and Harris). Targets present themselves at different stages of the metastatic process (Fig. 4) and may be features of the tumour cells or of the cells with which they interact. An obvious advantage to targeting those interacting normal cells which determine the success of metastasis is that these targets lack the inherent genetic plasticity of the tumour cells. They are, thus unlikely to evolve resistance to the agents used to inhibit them although, of course, the tumours could acquire reduced dependency on interactions with these cells.

These novel approaches are exemplified by attempts to target tumour neovasculature (Deplanque and Harris). Moreover, since many of the attributes required of

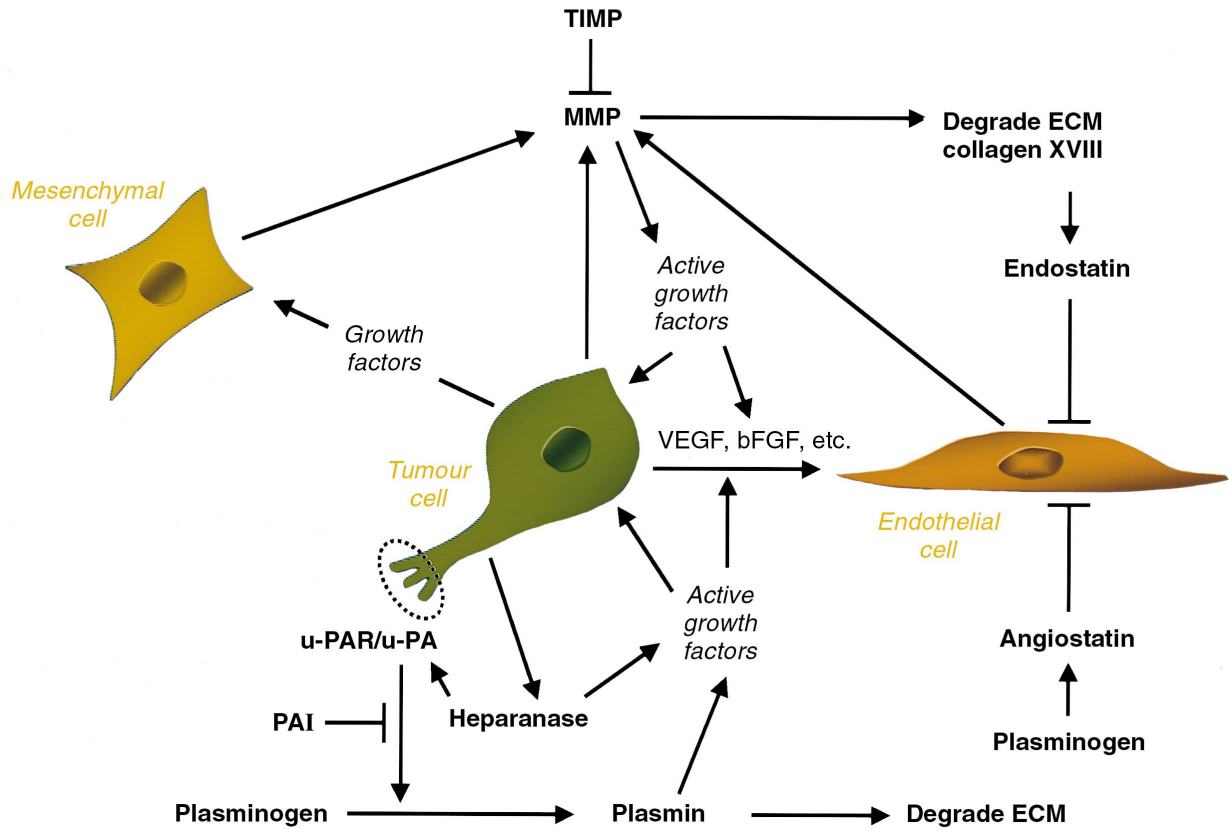

Fig. 3. Some of the interactions between tumour, stromal mesenchymal and endothelial cells that influence metastasis. Positive interactions are shown by arrows (→), negative by blocked lines (—⊣). See text for abbreviations and other contributions for further details.

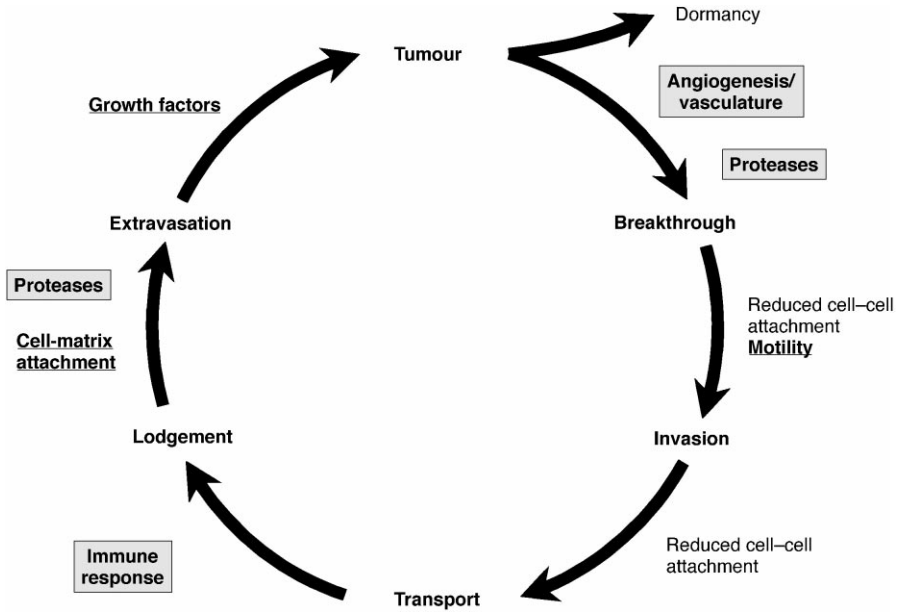

Fig. 4. Potential targets for breaking a vicious circle. The metastatic process is depicted as a circle, as in Fig. 2, with the addition of features important at different stages. Features shown in grey boxes represent targets for therapeutic intervention that have reached the stage of clinical trials (see articles in this volume; immune therapy has not been covered here). Features underlined are those whose targeting is still mainly at a preclinical experimental stage.

angiogenic endothelium are also needed by invasive tumour cells, targeting these features in the former may also directly attack the latter. This is, for example, the case for anti-adhesive peptides, with proposed uses in inducing apoptosis or in targeting drugs [2] and also for protease inhibitors (Curran and Murray). Anti-angiogenic therapies clearly show some promise (Deplanque and Harris) but, to realise their potential, we need a more complete understanding of the process that is being targeted. Thus, the paradoxical properties of MMPs, referred to above, are reflected in polarised possibilities for exploiting proteolysis for therapy; while conventional wisdom would favour inhibiting proteolysis, Reijerkerk and colleagues argue the case for enhancing it as a therapeutic option.

It is also clear that treatments aimed at the host response to the tumour, rather than at the tumour itself, will require novel means of assessing efficacy (Deplanque and Harris, Curran and Murray), since tumour dormancy rather than tumour elimination is a likely end-point. Increasingly sensitive and discriminatory techniques for tumour imaging (Glasspool and Evans, pp. 1661–1670) will, therefore, be needed, not only for diagnosis but also for monitoring therapy.

Some years ago I chaired a working group of the United Kingdom Cancer Research Campaign, charged with encouraging more and better research into metastasis. At that time, research appeared to fall into two discrete categories; laboratory studies aimed at understanding the metastatic process and clinical investigations seeking to improve treatment of disseminated cancer. The two areas seemed to have little common ground. Indeed, some clinicians explicitly stated that they could not see how understanding the process would help in managing patients presenting with tumours that had already metastasised. As the contributions to this volume demonstrate, that situation has changed radically, with new and promising treatments firmly based on an understanding of the processes of invasion and metastasis and clinical and basic researchers shooting at the same goal. The consensus would now agree with the conclusion of the article by McLeod and colleagues, that "understanding the biological pathways which regulate the molecular signature of metastatic disease offers great promise for therapeutic outcome for patients".

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