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Metastasis: recent discoveries and novel treatment strategies

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Abstract

Most cancer deaths are due to the development of metastases, hence the most important improvements in morbidity and mortality will result from prevention (or elimination) of such disseminated disease. Some would argue that treatments directed against metastasis are too late because cells have already escaped from the primary tumour. Such an assertion runs contrary to the significant but (for many common adult cancers) fairly modest improvements in survival following the use of adjuvant radiation and chemotherapy designed to eliminate disseminated cells after surgical removal of the primary tumour. Nonetheless, the debate raises important issues concerning the accurate early identification of clonogenic, metastatic cells, the discovery of novel, tractable targets for therapy, and the monitoring of minimal residual disease. We focus on recent findings regarding intrinsic and extrinsic molecular mechanisms controlling metastasis that determine how, when, and where cancers metastasise, and their implications for patient management in the 21st century.

Introduction

Metastasis is the culmination of neoplastic progression. In their classic review, Hanahan and Weinberg describe six hallmarks of cancer.¹ Besides immortality, abnormal growth regulation, self-sufficient growth, evasion of apoptosis, and sustained angiogenesis, invasion and metastasis are identified as distinguishing characteristics. However, although invasion through the basement membrane is the hallmark that objectively defines malignancy, not all neoplasms are invasive (eg, ductal carcinoma in situ of the breast and prostatic intraepithelial neoplasia), although they can progress towards malignancy. Similarly, the ability to metastasise is not an inherent property of all neoplastic cells. Some tumours are highly aggressive, forming secondary lesions with high frequency (eg, small cell carcinoma of the lung, melanoma, pancreatic carcinoma) whereas others rarely metastasise to distant sites despite being locally invasive (eg, basal cell carcinomas of the skin, glioblastoma multiforme).

Metastasis is generally described in terms of haematogenous (bloodborne) dissemination. However, secondary tumours can arise via spread through lymphatics (lymph node metastasis is a common feature of many carcinomas) or across body cavities (eg, ovarian carcinomas mainly establish secondary tumours by dissemination within the abdomen, rarely forming metastases via haematogenous spread). Cells can even migrate along the spaces between the endothelium and basement membrane or along neurons, as is the case in pancreatic carcinomas. The molecular and cellular mechanisms underlying these different proclivities are the topic of

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Conflicts of interest statement

We declare that we have no conflict of interest.

constant debate² and intense research efforts because they have important implications for our ability to predict, identify, and eradicate life-threatening metastatic disease.

Search strategy and selection criteria

We tried to identify all relevant studies irrespective of language. We searched PubMed, Medline, and Current Contents with a combination of the following terms: “metastasis”, “invasion”, “dissemination”, “chemokine”, “gene”, “protease”, “angiogenesis”, “lymphangiogenesis”, “signalling pathways”, “stroma”, “hypoxia”, “stem cell”, “host”, “gene signature”, “cell adhesion”, “extracellular matrix”, “microenvironment”, and “cancer therapy”. Studies were selected on the basis of recent contributions to the understanding of the cellular and molecular basis of cancer metastasis. Most searches covered 2000–07, but earlier papers were also included. Further references were selected from the bibliographies of cited papers.

Basic mechanisms of metastasis

Progression towards an invasive phenotype

The process of metastasis begins before cells migrate from a primary tumour mass. Among the earliest characteristics of transformed cells are genetic and phenotypic instability. Cancer cells are more prone to mutation and phenotypic drift than their normal counterparts.^{3–5} Genetic instability, coupled with a Darwinian type of selection—survival of the fittest—results in populations resistant to normal homeostatic growth controls, immune attack, and environmental restraints.⁶ The rate of progression varies and, within any neoplastic mass, subpopulations can be isolated with different malignant potential (figure 1). Thus, not all tumours are metastatic, nor are all cells within so-called metastatic tumours capable of metastasising.^{7–9} Even cells isolated from large metastases show substantial heterogeneity when assessed experimentally,¹⁰ raising questions as to whether cells might transiently acquire metastatic potential.⁶

Recent evidence suggests that tumour cells might begin conditioning distant tissues for colonisation by establishing a so-called pre-metastatic niche.¹¹ As yet unknown factors mobilise haematopoietic stem cells to tissues, remodel the matrix, and modify stromal cells and the growth factor milieu such that tumour cells are attracted to or have increased predilection for growth at these sites.¹² In a transgenic mouse colon carcinoma model, CD34+ immature myeloid cells expressing the chemokine receptor CCR1 were recruited from the bone marrow to the edges of local primary lesions and stimulated local invasion by tumour cells expressing the ligand CCL9.¹³ Importantly, genetic instability, generation of variants, and establishment of premetastatic niches represent intrinsic tumour cell and microenvironmental changes that take place before cancer cell dissemination.

Epithelial-mesenchymal transition

Neoplastic cells might acquire the ability to metastasise by dedifferentiation to a more motile mesenchymal cell phenotype, a process called epithelial-mesenchymal transition (EMT).^{14, 15} Once established in a new environment, metastatic cells might then revert back to a non-metastatic phenotype, via a mesenchymal-epithelial transition. Epithelial-mesenchymal transition can be induced by different stimuli, with transforming growth factor (TGF) β signalling having a key role. Other important mediators include oncogenic signalling pathways (notably phosphoinositide 3 [PI3] kinase), mitogen-activated protein (MAP) kinases, loss of E-cadherin (or a switch to N-cadherin), and activation of transcription regulators such as Twist and Snail (SNA1).^{16–18} Interestingly, Wnt, Notch, and Hedgehog signalling pathways (also implicated in stem cell maintenance) are linked to epithelial-mesenchymal transition.¹⁹

Cells induced to undergo epithelial-mesenchymal transition not only exhibit enhanced motility but also are resistant to apoptosis: key requirements for successful metastasis.²⁰ However, other cancer cells might use a collective migration that is independent of an epithelial-mesenchymal transition.²¹ The fact that Wnt signalling can also induce collective migration in addition to epithelial-mesenchymal transition emphasises the complex interrelations and plasticity in all of these processes.²²

Although a role for epithelial-mesenchymal transition during development is well accepted and can be demonstrated and manipulated in many experimental tumour models, some question whether it occurs in human cancers,²³ and it is important to state explicitly that epithelial-mesenchymal transition is not synonymous with invasion or metastasis.

Resistance to apoptosis and anoikis

Dissemination requires that tumour cells detach from the matrix or cell-cell anchor(s) that control tissue architecture. Under normal circumstances, epithelial cells undergo apoptosis (programmed cell death) when adhesion to the correct substrate is disrupted.^{24,25} Indeed, a specialised form of apoptosis—anoikis—results when normal cells are maintained in suspension; this process is clearly a mechanism designed to protect multicellular organisms from rogue cells establishing themselves outside their correct anatomical location. Metastatic cells therefore must be resistant to anoikis and apoptosis to survive during dissemination and colonisation of ectopic sites. Many studies show that crucial apoptotic modulators are deregulated in metastases. This deregulation is accomplished by various means: activation of survival pathways (eg, PI3 kinase-Akt), upregulation of matrix metalloproteinases (which downregulate death receptors, release growth factors, and condition the extracellular matrix for invasion); overexpression of anti-apoptotic proteins (BCL-2, BCL-XL) or focal adhesion kinase (FAK), and inactivation of p53, among others.²⁶⁻²⁸ The importance of anoikis resistance in metastasis was elegantly shown in experimental studies where a functional screen for suppressors of anoikis identified the neurotrophic receptor TrkB as a key mediator. Rat intestinal epithelial cells are very sensitive to detachment-induced anoikis and are non-tumourigenic, but when transfected with TrkB, they become highly tumourigenic and metastatic via both the lymphatic and haematogenous routes, even destroying bone.^{29,30} TrkB is often overexpressed in human malignancies and is mutated in colon cancer.³¹ Its activation also induces vascular endothelial growth factor (VEGF) expression via hypoxia inducible factor (HIF) 1 α , potentially assisting with establishment and angiogenesis of tumours at secondary sites.³²

Angiogenesis and lymphangiogenesis

That tumour growth and progression is limited before vascularisation of the neoplastic mass is generally accepted.³³ Vascularisation is achieved via neo-angiogenesis,³⁴ co-option of existing blood vessels,³⁵ vasculogenic mimicry (in which poorly differentiated, highly malignant tumour cells can form a primitive vascular system),³⁶ or a combination of these processes. Newly formed leaky capillaries can also serve as conduits for disseminating cells.

Hypoxia and activated oncogenes, including *RAS*, *EGFR*, and *HER2/NEU*, upregulate angiogenic cytokines (eg, VEGF and interleukin 8) and proteolytic enzymes (eg, matrix metalloproteinases, urokinase plasminogen activator [uPA]) and downregulate inhibitors such as thrombospondin (TSP1) via PI3 kinase and MAP kinase signalling pathways, thus potentiating angiogenesis, tumour growth, and spread.^{33,37,38} Hypoxia could also directly affect tumour cell motility, invasion, and metastasis, with a key component recently identified as HIF1 α -regulated lysyl oxidase (LOX).³⁹ LOX is linked to poor prognosis in several tumour types, including breast and oral cancers. It is thought to regulate FAK activity, cell-matrix adhesion, and motility, potentially creating a niche permissive for metastatic growth at

secondary sites. CXCR4, a chemokine implicated in site-selective metastasis, is also upregulated by hypoxia as well as oncogenes such as *HER2*, *MET*, and *EGFR*.⁴⁰⁻⁴² Hypoxia regulates many other genes linked to tumour progression,^{43,44} recruits macrophages and other inflammatory cells,⁴⁵ and can also contribute to increased genetic instability^{46,47} and resistance to apoptosis.⁴⁸

A parallel process—lymphangiogenesis—has been invoked as a potential facilitator of lymphatic metastasis,^{49,50} although functional lymphatic vessels within human tumours are rare and co-option of existing lymphatic vessels could also occur.⁵¹ The major lymphangiogenic cytokines (VEGF-C and VEGF-D) and lymphangiogenesis have been linked to poor prognosis in some cancers, and more specifically with lymph node metastasis.⁵²⁻⁵⁴ Experimental manipulation of these cytokines modulates lymphatic metastasis in some experimental models.⁵⁵⁻⁵⁸ VEGF-A⁵⁹ and other signalling systems—eg, angiopoietin:Tie, ephrin:Eph, and PDGF-BB:PDGFR—could also be involved.⁶⁰ Recently, so-called zip codes have been identified on the lymphatic endothelium in tumour xenografts which, when blocked, inhibited lymphatic metastasis.⁶¹

Two questions are of particular interest: can the propensity for lymphatic metastasis be predicted from gene expression signatures, as has been claimed for other sites of metastasis? And does lymphatic dissemination predispose to distant metastasis? The role of lymphatic dissemination has recently been reviewed,⁶²⁻⁶⁴ and that nodal metastases could serve as a bridgehead for further dissemination in certain cancers is clear; however, direct haematogenous dissemination occurs in others. Clearly, more careful kinetic and molecular dissection of cancer spread is required to delineate the importance of tumour cells detected not only in nodes, but also in the blood and bone marrow.^{63,65-67}

Dissemination and colonisation of secondary sites

Several million cells per gram of tumour can be shed daily into the lymphatic system or bloodstream.⁶⁸ The fate of bloodborne tumour cells is somewhat controversial and experimental evidence contradictory. In some models, most circulating cells die,^{69,70} whereas in others, most survive and extravasate.⁷¹ Insufficient data exist to quantify the fraction of shed tumour cells that successfully seed secondary tissues, especially in human cancers. Nevertheless, all studies show that most cells entering the vasculature fail to form macroscopic foci at distant sites.

What, then, is required of a cell to successfully colonise another tissue? These abilities must co-exist within a single cell since metastases are mainly clonal.^{72,73} To accomplish this process, tumour cells use a variety of motility mechanisms,^{74,75} chemokine gradients,⁷⁶ and proteinases (eg, matrix metalloproteinases, cathepsins, uPA, etc)⁷⁷⁻⁸⁰ to enter and exit the circulation. Many of the processes are also activated by endothelial cells during angiogenesis.^{81,82} Interestingly, Friedl and colleagues⁸³ recently showed that the migration of tumour cells through collagen matrices still occurs in the presence of broad-spectrum proteinase inhibitor cocktails. Additionally, the intuitive assumption that these enzymes are derived from tumour cells has been challenged by the finding that most are produced by stromal cells.^{80,84,85}

Ultimately, metastatic cells must lodge at secondary sites and re-establish adhesive connections. During haematogenous dissemination, the transit time is only seconds, thus cells are unlikely to shut down transcriptional expression of adhesion molecules as they depart the primary tumour and re-express them when they arrive at secondary sites. Cells could use alternative adhesion molecules or might selectively alter adhesion by post-translational modification of already expressed proteins, glycoproteins, lectins, or other molecules.⁸⁶

Metastatic cancer cells must also evade immune effectors or co-opt immune/inflammatory cells to assist them in completing subsequent steps of the metastatic cascade, and they must resist hydrostatic shear forces (ie, turbulence within vessels). Susceptibility to such stresses can vary widely and could contribute stochastically to metastatic inefficiency. Nonetheless, a successful metastatic cell must overcome whatever challenges to its survival are mounted.

Key issues

When and how is metastatic potential determined?

Gene expression microarrays have provided hope for the vexing issue of identifying which tumours will or will not metastasise. Chemotherapy or hormonal therapy reduces the risk of distant metastases by about a third; however, 70–80% of breast cancer patients receiving adjuvant treatment would have survived without it. Because these patient populations cannot be accurately identified, they are treated unnecessarily.⁸⁷ Several research groups have used microarrays to identify so-called poor prognosis markers, molecular portraits, or death from cancer signatures that show promise for segregating patient subsets with different phenotypes, with the ultimate aim of customising treatment according to need⁸⁷⁻⁹³ (table 1). A recent prospective, randomised study comparing conventional clinicopathological criteria with the 70 gene signature—a set of 70 marker genes that predicts poor prognosis⁸⁷—has been set up to select patients for adjuvant chemotherapy. This study is called Microarray In Node negative Disease may Avoid Chemotherapy (MINDACT; EORTC Protocol 10041-BIG 3-04).

Gene expression array data, in addition to providing promising prognostic and predictive tools, have also contributed to an important debate regarding the molecular basis of metastasis. The poor prognosis pattern of gene expression has been observed in primary tumours,^{90,93} suggesting that acquisition of metastatic competence is an early event (ie, hardwired) in tumour progression. This observation conflicts with the hypothesis that metastatic competence is due to the late emergence of specialised subclones (figure 1). How do we reconcile these two sets of observations? First, since tumours are heterogeneous, it could be that the metastasis signature is represented by the bulk population, but not by every cell within that population (ie, the expression is distributed among all the cells). Some subpopulations within the tumour could express one of the metastasis signature genes, whereas others could express larger proportions (or all) of the signature genes. Since metastases are clonal, successful cells must individually express all required properties, or be able to depend upon host support for those they lack. Second, two types of profiles have been identified by Minn and colleagues:¹⁰² a general metastagenicity set of genes (which coincidentally also potentiated primary tumour growth), superimposed upon which are (probably fewer) expression changes linked to metastasis per se, and what is more, metastasis at specific sites: organ virulence genes.¹⁰² Thus, genes relating to angiogenesis, survival, and proliferation (required for both primary and secondary tumour growth) could skew the expression profiles towards a high degree of concordance. Recently, a novel hypothesis has been proposed: that disseminating cells might return home to the primary site, increasing the population of cells in the primary tumour with metastatic molecular profiles.¹⁰⁸ However, in other cancer types, malignant cells in bone marrow (and other sites) can show substantial genetic differences compared not only with the primary tumour, but also with each other, suggesting early (or sequential) dissemination and parallel evolution.¹⁰⁹ Clearly there are implications from both of these (non-exclusive) mechanisms of metastasis for clinical management: in the first case, sampling the primary tumour could adequately predict the make-up of metastases, whereas if they have been generated independently, even determining the expression profile of one metastasis might not reflect that of others. Since new treatments now aim to inhibit specific underlying oncogenic pathways in cancer (eg, due to Bcr-Abl translocations, activated tyrosine kinases, mutant proteins), it will be important to be able to predict with some degree of certainty the prevalence of the target within disseminated disease sites to select patients for therapy and to assess response.

Several of the genes identified in metastasis signatures are stromal in origin,^{110,111} again attesting to the contribution of the host to metastasis.¹¹² Many of these genes are concerned with remodelling of the extracellular matrix, invasion, and motility, in addition to those required for survival and proliferation. Doubt has been cast on their functional importance since there is little concordance in the genes identified in different studies.¹¹³ However, a recent editorial¹¹⁴ has suggested that many of the reported gene signatures are equally predictive and that combining them could be even more powerful. That the complementary functions required for successful metastasis could be fulfilled by different genes in different cancers is possible. The myriad transcriptional changes observed could be orchestrated by a few key functional regulators. For example, the wound response signature (a powerful predictor of metastasis in multiple tumour types)¹¹⁵ is induced by coordinated amplification of two genes, *MYC* and *CSN5*.¹¹⁶ Their overexpression was sufficient to induce rapid cell growth and invasiveness, but required cooperation of other genes for initial oncogenic transformation. Others have identified a metastasis expression signature similar to that of stem cells.^{88,117}

What determines site selectivity of metastases?

Disseminated cells colonise certain tissues more commonly than others¹¹⁸ (figure 2). Some preferred locations can be accounted for by blood flow (eg, liver from colorectal carcinomas, vertebrae from prostate carcinomas), whereas others cannot (eg, liver from uveal melanoma, long bones from breast carcinoma).^{119,120} Paget explained¹¹⁸ this non-random distribution by suggesting that the seed (tumour cell) will only grow in an appropriate soil (tissue microenvironment). Numerous studies support this notion and several mechanisms might contribute: tumour cells could bind selectively to endothelial cells or basement membranes from certain tissues, invade more readily, or respond to organ-specific growth factors depending on the receptors expressed and signalling pathways engaged.^{121,122} The ERB-B/HER family of receptor tyrosine kinases: epidermal growth factor receptor (EGFR/ERB-B1, ERB-B2 [HER2], ERB-B3, and ERB-B4) are often overexpressed and, in some cases, mutated in human cancers. Upon ligand binding, they form homodimers or heterodimers and strongly activate the MAP kinase and PI3 kinase signalling pathways among others, leading to cell proliferation, motility, and invasion. MET, another receptor tyrosine kinase, and its ligand, hepatocyte growth factor, are also often upregulated in cancers. The predilection of breast cancer cells expressing ERB-B oncogenes to generate CNS metastasis^{123,124} could be explained by the fact that their cognate ligands (heregulins/neuregulins) are brain growth factors; similarly colon or pancreatic carcinoma cells overexpressing EGFR or MET could respond, respectively, to high levels of TGF α or hepatocyte growth factor in the liver.^{125,126}

Recently, the Massagué group used cDNA microarrays to compare lung, bone, and adrenal colonising breast carcinoma cell lines.^{72,102} They found that some genes expressed by tumour cells were common for metastases to all sites (eg, osteopontin), whereas others were selectively expressed if the cell lines had a predilection to growth in a given tissue (eg, CXCR4). Importantly, they found that combinations of genes were required for successful metastasis to each site; a single molecule was not sufficient. Other site-specific gene signatures have been reported in both experimental models and clinical samples (table 1).

Several clear examples of gene expression profiles relate to bone metastasis (again superimposed on a high-risk signature),^{72,96,102} perhaps because this site represents a more specialised and demanding environment than soft tissues. There is evidence for osteomimicry, with successful metastatic cells responding to bone-derived chemotactic and mitogenic factors and entering a vicious cycle of bone degradation and remodelling.¹²⁷⁻¹²⁹ Briefly, the vicious cycle develops when factors secreted by or expressed on tumour cells (eg, parathyroid hormone-related peptide) activate osteoblasts and osteoclasts in the bone micro-environment to produce cytokines (eg, receptor activator for nuclear factor κ B ligand, RANKL); by contrast,

osteoprotegerin is downregulated. Remodelling and osteolysis causes release of growth factors (eg, TGF β and insulin-like growth factor [IGF] 1), which then stimulate tumour cell growth and motility and further release of parathyroid hormone-related peptide. Molecular profiling might help to identify patients who could benefit from bisphosphonates or other agents that interfere with bone colonisation.

Examination of bone marrow metastases identified different determinants, with evidence that this site might predict for later distant relapse.⁹⁷ Early dissemination also seems to indicate seeding of cells of low metastatic potential which can remain dormant for extended periods. To survive and thrive, they need to adapt or be reinforced by later waves of better-equipped cells. Identifying which of these cells are genuine invaders is of major clinical significance.

Whether lymphatic metastasis is an active process, or merely due to the ease of access of tumour cells to lymphatic vessels and passive transport to draining nodes, is not clear. Several studies have identified gene expression profiles that predict lymphatic metastasis,^{101,130,131} whereas others have not.¹³² Interestingly, Hoang and colleagues⁹⁸ suggested that the molecular signature of nodal metastasis was a composite of two patterns of gene expression: one that was similar between primary cancers and metastases, and a small subset of 27 genes that discriminated metastases, perhaps offering an elegant solution to the debate between clonal selection versus predeterminism.

What are the genes that control metastasis?

Identification of specific metastasis genes is difficult because of the need for several complementary functions that might be fulfilled by different genes in different contexts. However, several laboratories have identified more than 20 metastasis suppressors (table 2) that inhibit metastases without blocking tumour formation.¹³³⁻¹³⁷ The existence of such suppressors argues against metastatic potential being completely hardwired into cancer cells. Interestingly, metastasis suppressors seem to be involved in several pivotal positions that would amplify key molecular signals. Many are effective in tumour cells of multiple tissue origins, suggesting common pathways for metastasis regulation and control.

Recently, the metastasis suppressor gene *CD82/KAI1* has been shown to interact with a protein (DARC; Duffy antigen receptor for chemokines, also known as gp-Ly) on endothelial cells and induce senescence of tumour cells, indicating a role in preventing ectopic dissemination and growth.¹⁴⁰ Many metastasis suppressors block growth of cells at secondary sites.^{136, 138,139} For example, when metastasis competent C8161 human melanoma cells or their metastasis-suppressed counterparts that express neo6/C8161 or KISS1 were injected intravenously into immunocompromised mice, the parental and metastasis-suppressed cells distributed equally well throughout the body.¹⁴¹ The number of microscopic foci in various organs initially diminished equally; however, only the metastatic C8161 cells were subsequently able to proliferate in the lung environment to form progressive, lethal tumours. By contrast, cells expressing neo6/C8161 and C8161-KISS1 persisted for several months either as single cells or clusters of fewer than ten cells. These dormant cells could be isolated from the lungs, established in tissue culture, and were tumourigenic when transplanted at the normal position (intradermal site). From this location, they were also able to seed to the lungs but failed to develop into overt metastases. In short, cells expressing the metastasis suppressor gene were capable of completing every step of the metastatic cascade except proliferation at the secondary site.¹³⁹

These observations suggest that at least some metastasis suppressors are mediators of cellular context and might regulate metastasis to some sites, but not others. However, the molecular mechanisms underlying differential growth remain ill defined. In addition to being mediators of microenvironmental signals, expression of metastasis suppressors is also subject to

extracellular cues. Early analyses show that these genes (by contrast to many tumour suppressors such as p53, adenomatous polyposis coli gene [APC], and phosphatase and tensin homolog [PTEN]) are not often mutated, but more commonly controlled by epigenetic mechanisms.^{139,142}

Microenvironmental issues: cellular context and tumour-host interactions

That metastatic potential is not simply an inherent trait of cancer cells, but is substantially modified by the microenvironment, is clear.^{112,143,144} The pioneering studies of Bissell and colleagues¹⁴⁵ showed that even fully malignant breast cancer cells could be reverted to a normal phenotype by exposing them to a non-permissive stroma;¹⁴⁵ conversely, cells could acquire malignant potential at sites of wounds or irradiated tissue.¹⁴⁴ The extracellular matrix of secondary sites such as bone could also potentiate survival of metastatic prostate and breast carcinoma cells.^{146,147} Tumour cells can themselves induce a permissive environment and might even provide selective pressure for stromal cell mutations—eg, loss of functional p53¹⁴⁸—in addition to inducing proteolytic enzymes in stromal macrophages and fibroblasts, and stimulating angiogenesis.

Two important papers provide examples of how tumours might condition their environment to sustain their development. Topczewska and colleagues¹⁴⁹ used zebra fish as a biosensor and found that malignant melanoma cells re-programmed the phenotype of adjacent cells to ensure a biological niche in which they could thrive; this was mediated by secretion of a potent embryonic morphogen, Nodal. Inhibition of Nodal prevented invasion and reverted the cells towards a melanocytic phenotype. Kaplan and co-workers¹¹ found that primary tumours can precondition future metastatic sites. Factors released by the primary tumour induce VEGFR1-positive bone marrow haematopoietic progenitor cells to home to specific tissue sites and form cellular clusters before arrival of the tumour cells, perhaps providing a permissive niche akin to that required by stem cells. We are now beginning to decode the language used in the cross talk between tumour cells and their environment that could lead to eventual interruption of this dialogue.

Important experimental data from Kent Hunter's group suggest that metastatic propensity might be affected by host genetic context.¹⁵⁰ Briefly, transgenic mice of FVB/NJ strain expressing the polyoma middle T oncogene (PyMT) develop metastatic mammary tumours with a high incidence. However, when crossed with other inbred strains, metastatic potential is either enhanced or inhibited. Since all tumours are initiated by the same oncogenic event, these differences are most reasonably explained by inherited susceptibility. Indeed, *Sipa1* (a Rap1GAP gene) has been identified recently as a polymorphic metastasis modifier gene in this model. Its expression level is also linked to metastasis in human prostate cancers.¹⁵¹ Of great interest is the fact that, in the PyMT transgenic tumour models, the 17-gene metastasis signature described by Ramaswamy and colleagues⁹⁰ was evident in tumours from the high-incidence mouse strains, and furthermore, some subsets of metastasis-predictive gene expression patterns are evident in the normal tissues of these animals.¹⁵⁰

The intriguing possibility exists that signatures that are predictive of metastasis lurk in our genomes and that constitutional genetic variation substantially modulates metastatic efficiency.¹⁵² Whether such a profile exists (or could be deciphered) in outbred human populations remains to be seen, but clearly host genetic and epigenetic environmental effects cannot be ignored. The spread of cancers apparently occurs with a frequency that is inconsistent with the necessity for additional mutational events¹⁵³ and hypermethylation of suppressor genes is also a key regulator.¹⁵⁴ Chen and colleagues¹⁵⁵ found that a murine tumour cell line exhibited a bone-metastatic phenotype in syngeneic strain 129 mice, but this was shifted to liver metastasis in a different host strain, although the mechanisms have not been elucidated yet. Thus, although classic theory has considered the seed and the soil, we should also now recognise that host

“climate” is another important determinant in metastatic growth. The various (non-exclusive) mechanisms are illustrated in a simple form in figure 1.

When is a metastasis not a metastasis?

Observations that tumour cells behave differently depending upon their microenvironment highlights the importance of clearly defining the tumour-host signalling pathways and circuitry involved, and also raises two important and practical questions: what is the clinical relevance of disseminated cells, and are single disseminated cells metastases?

The presence of single ectopic cells was not an issue until techniques capable of detecting them (or small emboli) were developed. Indeed, happenstance sections in tissues often detect disseminated neoplastic cells, but their clinical importance was negligible since they were not affecting function. This observation does not belittle the potential clinical importance of these cells, since they have accomplished most of the antecedent steps of metastasis. The potential to become cancerous is present, but when they blend innocuously, they are difficult to detect and neutralise. The challenge is to discriminate between those cells merely leaning toward such behaviour from those that are destined to become cancerous. Nonetheless, accumulating evidence suggests that the presence of tumour cells in the peripheral circulation, lymph nodes, or bone marrow are indicators of poor prognosis; however, the correlations are imperfect.⁶⁶ Recent staging criteria now incorporate parameters of single cells or microscopic masses.
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The question as to whether isolated disseminated cells should be deemed to be metastases is still debated. In 1919, James Ewing¹⁵⁹ referred to an uncited study by M Schmidt thus:

“From the study of lung in these cases [of cancer], Schmidt concludes that in cancers of the abdominal organs, there is frequent and repeatedly a discharge of cancer cells which lodge in the small arteries of the lungs [but fail to form metastases]”

Presently it is not known whether the persistent cells are dormant (ie, non-dividing) or of limited replicative ability. Dormancy would explain resistance to treatments that, for the most part, target proliferating cells; by contrast, a limited replicative ability would provide a potential mechanism for new mutations being passed to progeny. Failure to develop a blood supply has been suggested to be a potentially important mediator of tumour dormancy for which there is compelling experimental evidence.¹⁶⁰ However, other mechanisms such as the action of tumour suppressor genes¹⁶¹ or immunological restraint¹⁶² might also have a role. Conversion to an actively proliferating population could also occur in response to changes in the host tissue (eg, injury, change in hormonal status, ageing, initiation of angiogenesis) or the development of a suitable niche.¹⁶³

Failure to distinguish bona fide metastases from inert disseminated cells has important implications. Current medical practice is to eliminate all risk. Therefore, if cells have already spread, then aggressive treatments are advocated. Also, the extent and location of spread (ie, local vs circulating vs regional vs distant) determine the treatment plan. However, patients might be subjected to more cytotoxic agents than necessary since most disseminated cells fail to form secondary tumours. In addition to microarray data, several investigators have queried the relevance of circulating cells with regard to their prognostic and predictive value. This debate is valid and is the subject of prospective clinical trials.¹⁶⁴ Interestingly, in many breast cancer patients (including several with HER2-negative primary cancers), HER2-positive circulating cancer cells were identified.¹⁶⁵ This finding, together with the well-validated concept of micrometastases in breast cancer, could contribute to the success of the use of the anti-HER2 antibody trastuzumab in combination with adjuvant therapy after surgery.^{166,167}

The distinction between single ectopic cells versus metastases also has ramifications for assessing efficacy in clinical trials. Does prevention of secondary outgrowths constitute a successful anti-metastatic treatment, or must all cells be eliminated? These topics have been actively debated even before the first clinical trials have begun.^{71,168}

Molecularly targeted treatments

Classic treatments for disseminated disease—ie, radiotherapy or chemotherapy—are mainly aimed at rapidly proliferating cells via inhibition of DNA replication, DNA repair, or the cell cycle. We are now in an era of targeted treatments based on the molecular pathology of the cancer in question, and although there have been some notable successes of agents targeting oncogenic signalling pathways (eg, imatinib, trastuzumab, erlotinib, gefitinib), there is still a way to go.¹⁶⁹⁻¹⁷² Nevertheless, we now have an impressive armamentarium of both small molecule inhibitors and antibodies to many oncogenic receptor tyrosine kinases, and efforts are underway to define the best way of combining these agents for maximum therapeutic benefit. The next generation of treatments will focus on supplementing these approaches by addressing new aspects of malignant behaviour.

The first anti-angiogenic agents are now being tested in the clinic, for example bevacizumab (an anti-VEGF antibody^{173,174}), vascular disrupting agents such as combretastatin A4,^{175,176} and several other small molecules, antibodies and soluble decoys that target angiogenic growth factor receptors (VEGFR-2, VEGFR-3, PDGF-R, FGFR-2). Additionally, inhibitors of HIF and downstream signalling pathways show promising results.^{81,177} Recently, treatments combining anti-angiogenic and cytotoxic agents were shown to reduce the tumour stem cell-like fraction in glioma xenografts,¹⁷⁸ indicating that this key population might be amenable to carefully designed therapeutic approaches.

There are also emerging strategies to reverse epigenetic events in oncogenesis—eg, aberrant histone methylation and acetylation.^{179,180} Several promising histone deacetylase inhibitors are in development,^{181,182} and it is thought that methyltransferases could also be targeted with drugs.¹⁸³ However, selectively reversing inappropriate DNA hypermethylation in the promoters of tumour or metastasis suppressor genes will be very challenging.

Other exciting new targets include the endothelin (ET1) receptor and RANKL (both implicated in bone metastasis) using antagonists such as atrasentan¹⁸⁴ and denosumab,¹⁸⁵ respectively.

Now is the time also to consider targeting molecular pathways in lymphangiogenesis,¹⁸⁶ anoikis,³⁰ and the mechanisms underlying tumour-host/stromal interactions and cell motility, especially since cell motility, more so than cell proliferation, distinguishes metastatic cancers from benign lesions.^{81,122} These approaches will require new paradigms in trial design and patient management¹⁸⁷⁻¹⁸⁹ (table 3). Many lessons have been learned from the disappointing trials with early generation matrix metalloproteinase inhibitors.⁸⁴ Contributing factors to the failures were the fact that patients with mainly late stage bulky cancers were treated and it is now clear that different matrix metalloproteinases can have both pro- and antitumour effects. The challenge now is to target specific matrix metalloproteinases while sparing those whose inhibition would be counterproductive¹⁹⁰ and carefully to select appropriate cancer patient populations.

Stem cells: are we shooting at the right cellular and molecular targets?

Complete eradication of metastases is rare. On the basis of our understanding of embryonic organ development and self-renewing adult tissues (notably the haematopoietic system), the idea is emerging that a unique population of stem cells might be responsible for generating both primary and secondary cancers, and that these cells are inherently resistant to standard

treatments. These concepts were first propounded by Cohnheim in 1875¹⁹¹ and later explored experimentally.^{192,193}

Adult pluripotent haematopoietic stem cells that can repopulate all of the blood cell compartments (and which, when they or their progeny become malignant, form leukaemias and lymphomas) are well described. However, the identification and characterisation of stem cells in epithelial tissues or the carcinomas that arise from them has been challenging, especially in human beings. Many cancers arise in tissues where self-renewal is essential (eg, gut, skin, bone marrow). In these tissues, a small number of stem cells, as well as maintaining their own numbers, also generate progenitor cells by asymmetric division. These progenitor cells undergo several rounds of proliferation before terminally differentiating. Only the rare stem cells are long-lived, probably as a means of limiting the accumulation of genetic damage. The default position is differentiation or death, and cancer occurs when this is overridden. The progenitor cells—in a caricature of differentiation—might be responsible for the heterogeneity evident in many cancers (and could contribute to differences in drug sensitivity and metastatic capacity). However, cytotoxic treatment could well kill the proliferating progenitor cells (reducing tumour burden) but fail to eradicate the stem cell compartment, and thus only rarely prove to be curative¹⁹⁴⁻¹⁹⁶ (figure 3).

So what is the evidence for stem cells in adult epithelial tissues and cancer, and what are their characteristics (panel 1)? Recently, two groups succeeded in repopulating an entire mouse mammary gland from a single cell, identifying this putative stem cell with a series of surface markers.^{197,198} Before this, Al-Hajj and colleagues¹⁹⁹ isolated a subpopulation of cells in human breast cancers with dramatically enhanced tumorigenic capacity when transplanted into immunodeficient mice. We do not know if cancer stem cells are derived from normal stem cells, but if so they might share one or more of their properties that would confer immortality and the ability to withstand genotoxic insults from the environment or, in the case of cancer cells, cytotoxic drugs. First, as part of their defence against damage, normal stem cells express several transporters (efflux pumps for drugs and toxins) that are the major multidrug-resistance genes in cancers.²⁰⁰ Additionally, stem cells have an active DNA repair capacity and exhibit resistance to apoptosis; furthermore, they are relatively quiescent, dividing rarely and leaving the expansion of the population to their progeny. All of these factors could contribute to the survival of stem cell cells able to repopulate a tumour or metastasis after cytotoxic drug treatment or radiation.²⁰¹ Recently, it has been postulated that migrating stem cells, located at the tumour invasive front, exhibiting epithelial-mesenchymal transition and an activated Wnt pathway, could be responsible for colon cancer metastasis.²⁰² Interestingly, this study, and others,^{74,203} have shown that motile cells are non-proliferating, again suggesting that new therapeutic modalities are required to target these dangerous cells.

Panel 1: Key properties of stem cells

- Capacity for self-renewal and progenitor production via asymmetric cell division
- Regulated by niche: does disruption of this host control lead to aberrant expansion of stem/progenitor cells and cancer?
- Long-lived: time to accumulate multiple mutations
- Ability to generate multiple lineages via downstream differentiation
- Active telomerase expression
- Activation of anti-apoptotic pathways (high Bcl-2 and IAP proteins)
- Anoikis resistance: survival during dissemination?
- Ability to migrate

- Increased membrane transporter activity: drug resistance
- Unique signalling pathway patterns: Wnt, Notch, Snail, Twist, Hedgehog, Bmi-1

Panel 2: Key unanswered questions

- What is the relation between metastatic cancer cells and stem cells? Are there really stem cells in solid human tumours? Do current anticancer drugs kill cancer stem cells? Will there be differences between malignant and normal stem cells?
- Are metastases the result of epithelial-mesenchymal transition? Does epithelial-mesenchymal transition contribute to progression of human cancers? Is there a transient metastatic compartment?
- Is metastasis an inherent (selectable) property of a subset of tumour cells or an adaptive property?
- What role does the host genotype have in determining metastatic risk?
- What are the mechanisms of action of metastasis suppressors? Are they part of a shared pathway or are there parallel mechanisms of metastasis control?
- What determines selective colonisation of different organs/tissues?
- Is the ability of tumour cells to proliferate in secondary sites the major rate-limiting determinant in metastasis?
- What is the relevance of circulating tumour cells (or tumour cells in the draining lymph nodes or bone marrow) with regard to establishment of distant metastases?
- Can microarrays or proteomics of tumours (or normal tissues) be used in the clinic to predict probability and/or sites of metastasis?
- How can early detection of metastases be improved?
- Is metastasis a tractable therapeutic option?
- What are the most promising molecular targets for preventing/treating metastases?
- How can we monitor minimal residual disease during therapy with a high degree of sensitivity and specificity?
- What are the molecular mechanisms responsible for tumour dormancy?

Do stem cells express the molecules that we are currently targeting? There are conflicting reports as to whether breast cancer stem cells express the oestrogen receptor and HER2, although EGFR is reportedly present. The major signalling pathways so far identified in stem cells are often mutated in cancers but they are not, by and large, the focus of current molecular treatments.²⁰⁴ Potential unexploited targets include TGF β , the Notch pathway^{205,206} (also implicated in angiogenesis²⁰⁷ and the osteomimetic properties of prostate cancers²⁰⁸), Wnt, Hedgehog, Snail, Twist, and Bmi-1.²⁰⁹⁻²¹¹

So, would targeting tumour stem cells damage normal stem cells? To answer this, we will need much better definition and characterisation of these two populations in a variety of tissues. However, recently Krivtsov and colleagues²¹² found phenotypic differences between stem cells in a mouse model of leukaemia compared with normal haemopoietic stem cells (a subset of which were also identified in human leukaemia), providing preliminary evidence that specific targeting of malignant cells might be feasible.

Conclusions and future prospects

Recent years have revealed exciting new insights into the molecular mechanisms of metastasis, although many questions remain (panel 2). We need to resolve the relative contributions of mutations in the seed cells (whether stem cells, their progeny, or both), epigenetic changes and microenvironmental influences, and not least inherited predisposition to cancer susceptibility and spread. Animal models have yielded important insights into the mechanisms of metastasis, but these must be measured against clinical reality. We need to develop more treatments that specifically target metastases, which must be tested in appropriate systems *in vitro* and *in vivo*. The design of clinical trials will be challenging: identification of patients at risk by genetic profiling and more sensitive methods of detection and quantitation of metastases are urgently required, together with early indicators of response. Prevention of metastatic outgrowth (eg, induced differentiation, stasis, or reactivation of apoptosis) is a further consideration.

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Figure 1. How and when is metastatic potential determined?

These mechanisms are not mutually exclusive, and could all contribute.

Figure 2.
Can cancer spread to different sites be predicted?

Figure 3.
Is failure to control metastasis due to residual tumour stem cells?

Table 1
Examples of gene signatures for cancer progression and metastasis

	Study	Outcome	Key genes/pathways	Reference
Clinical				
Risk of recurrence	Primary breast cancers	76 gene signature independently predicted metastasis in LN negative breast cancer patients [*]	CD44, cyclin E2	Wang et al ⁹⁴
Poor prognosis: breast	Primary breast cancers	70 gene "poor prognosis" signature in breast, 83% accuracy [*]	MMP1, MMP9, Flt-1 (also BUB1, VEGF)	Van't Veer et al ⁸⁷
Breast cancer subtypes	Primary breast cancers	Molecular portraits indicated five subsets		Perou et al ⁹⁵
Metastasis signature	Diverse metastases vs primary tumours	17 genes discriminated primary lung tumours with propensity to metastasise, correlated with poor survival	Many stromal genes: RUNX1, collagens, myosin	Ramaswamy et al ⁹⁰
Bone metastasis	107 primary breast cancers, LN negative	31 gene signature, 100% sensitivity, 50% specificity [†]	TFF1, TFF3, FGF-MAPK pathway	Smid et al ⁹⁶
Bone marrow metastasis	19 primary breast tumours	73 gene signature in LN negative patients	HIF1 α , CK 8, 18, 19, Jak-Stat pathway	Woelfle et al ⁹⁷
Lymph node metastasis	11 primary tumour and LN pairs from stage III NSCLC (excluded stroma)	27 gene set distinguished primary and secondary tumours	VAV3, PAX5, IGFBP7	Hoang et al ⁹⁸
Lymph node metastasis	Re-analysis of two previous array data sets of lung adenocarcinomas	318 gene set gave 94% in pN positive patients, but 21% in pN0 however these "false positive" patients had poor survival and possibly occult disease spread	Collagens, S100 calcium binding proteins	Xi et al ⁹⁹
Renal cancer metastasis	65 renal cancers, 24 normal kidney and ten metastases	155 gene signature predicted metastasis with 89% accuracy, also validates Ramaswamy signature in renal cell carcinoma	Caveolin 1, lysyl oxidase, annexin A4, MMP14, topoII α superimposed on angiogenic profile	Jones et al ¹⁰⁰
SCCHN lymphatic metastasis		116 genes predicted LNM	Transglutaminase 3, keratin 13, ERB-B4	O'Donnell et al ¹⁰¹
Experimental				
Bone metastasis	MDA MB 231 breast carcinoma injected into heart	102 genes segregated clones with bone and adrenal selectivity. 67% sensitivity, 80% specificity in patients [†]	CXCR4, FGF5 MMP1, ADAMTS1, IL11, CTGF, proteoglycan 1. Potentiated by TGF β and OPN	Kang et al ⁷²
Bone metastasis	MDA MB 231 breast carcinoma injected into heart	50-gene signature segregated patients with distant metastases to bone vs lung in breast cancer.	CXCR4, IL11, CTGF	Minn et al ¹⁰²
Lung metastasis	MDA MB 231 injected intravenously	54 gene signature (or 18 gene classifier) distinguished high or low risk of lung metastases in breast cancer	SPARC, VCAM1, ID1IL13R α 2, ID1, MMP1, MMP2, epiregulin	Minn et al ¹⁰³
Lymph node metastasis	PC3 prostate carcinoma orthotopic	No clinical validation reported	Cathepsins B, D, L, MMP16, integrin α 5 and α 6, uPAR	Chu et al ¹⁰⁴

	Study	Outcome	Key genes/pathways	Reference
Lung metastasis	MDA MB 435	No clinical validation reported, but some genes associated with poor prognosis	Integrin α 3, CD44, cyclin D1, vimentin, p53, XBP1, BST1	Bandyopadhyay et al ¹⁰⁵
Lymph node metastasis	MDA MB 435 orthotopic breast carcinoma comparing primary and secondary tumours	No clinical validation reported	CD73, integrin α 1 and β 5, HIF1 α and genes associated with tumour-matrix interactions	Lee et al ¹⁰⁶
Metastasis	PC3 orthotopic prostate and TRAMP transgenic models	11 gene “death from metastasis” (stem cell transcriptome) signature in multiple cancer types	BMI-1, Ki67, FGFR2, BUB1 etc, five of the 11 genes related to cell proliferation and mitosis	Glinksy et al ⁸⁹
Invasion	Array and proteomic profiles of MCF10a, MCF7, and MDA MB 231	Nine genes distinguished MCF7 and MDA MB 231	GPCR11, cadherin 11, vimentin, S100A8, LOX	Nagaraja et al ¹⁰⁷

BST1=bone marrow stromal cell antigen 2. CK=cytokeratin. CTGF=connective tissue growth factor. HIF=hypoxia-inducible factor. LN(M)=lymph node (metastasis). LOX=lysyl oxidase. NSCLC=non-small cell lung cancer. OPN=osteopontin. SCCHN=squamous cell carcinoma of the head and neck. TFF=trefoil factor (adhesion molecule). XBP1=X box binding protein.

*

Three gene overlap between studies.

†

One gene overlap between studies, but both implicated FGFR-MAPK pathway. One gene only (*BENE*) common to Smid⁹⁶ and Kang⁷² signatures, but five in the FGFR-MAPK pathway were all upregulated (*FGFR3*, *FGF5*, *SOS1*, *DUSP1*, *DUSP4*).

Table 2Examples of metastasis suppressor genes and their functions^{133,136,138,139}

	Function
Cadherin 11	Cell adhesion, cytoskeletal structure
Caspase 8	Pro-apoptotic enzyme
CD44	Cell adhesion (to hyaluronic acid); potentiates HER signalling
Claudins 1 and 4	Components of tight junctions between cells
CRSP3	Transcriptional coactivator/corepressor
Connective tissue growth factor	Integrin binding
Gelsolin	Actin polymerisation
E and N cadherin	Ca ²⁺ -dependent cell-cell adhesion
KAI1	Integrin interactions, EGFR desensitisation, binds DARC on endothelial cells
KISS1	G-coupled protein receptor ligand
RECK	Regulates matrix metalloproteinases
RhoGD12	Regulates Rho and Rac function in cell spreading and motility
Src-suppressed C kinase substrate	Modulates Rho signalling
JNKK1/MKK4	Activates p38 MAP kinase and/or JNK
MKK6	Activates p38 MAP kinase
MKK7	Activates JNK
Nm23	Histidine kinase, phosphorylates kinase suppressor of Ras (KSR), nucleotide diphosphate kinase activity
RKIP	Inhibits RAF-mediated MAP/ERK kinase phosphorylation
BRMS1	Chromatin remodeling, cell-cell signalling, PI3 kinase signalling
Drg-1	unknown

Table 3
Examples of current and future treatments for metastatic disease

Cellular functions	Examples of molecular targets	Examples of therapeutic agents in clinical or preclinical development
DNA replication	DNA, telomerase	Radiotherapy, alkylating agents, platinum drugs
DNA repair	Topoisomerases, PARP	Antimetabolites
Cell cycle	Tubulin, cyclins, aurora kinases	Anthracyclines, taxanes, VX-680
Metabolism, protein synthesis	RNA, thymidylate synthase	Antifolates, antimetabolites, tomudex, OSI-7904L
Oncogenic signalling	EGFR, ERB-B2, BCR-ABL, KIT, RET, PDGFR, mTOR, PI3K, RAF, COX2, PKC, farnesyl transferase, HSP90 chaperone, proteasome	Gefitinib, erlotinib, cetuximab, trastuzumab, imatinib, vandetanib [A1], RAD001, PI103, celecoxib, 17AAG, 17DMAG, bortezomib
Endocrine signalling	ER, aromatase, AR, CYP450c17	Tamoxifen, anastrozole, flutamide, abiraterone (Cougar Biotechnology)
Angiogenesis	VEGF, VEGF-R, integrins	Bevacizumab, semaxanib, vatalanib [A1], vitaxin [A1], combretastatins, endostatin, angiostatin
Proteolysis/invasion	uPA, matrix metalloproteinases	Amiloride, marimastat, prinomastat [A1], BMS-275291
Osteolysis	Farnesyle diphosphate synthase	Bisphosphonates (pamidronate, clodronate, zoledronate)
Drug-resistant phenotypes	BCR-ABL and EGFR mutations	Dasatinib, bosutinib (SKI-606, Wyeth)
Cell motility	C-MET, Src, ROCK, PLC γ , SDF1-CXCR4	PHA665752, AMD3100
Tumour-host interactions	TGF β , Slit-Robo, Eph-Ephrins, angiopoietins-Tie	
Osteoclastogenesis	RANKL, endothelin receptor	Denosumab (AMG162, Amgen)
Lymphangiogenesis	VEGFR-3	PTK/ZK
Hypoxia/glycolysis	HIF1 α , NF κ B, LOX	YC-1, vitexin [A1]
Epigenetic events	Acetylation, methylation	SAHA, LAQ824
Stem cells	Notch, Hedgehog, Wnt signalling pathways	Cyclopamine
Anoikis/survival	TRKB, PI3K, AKT; Twist, Snail, Bmi-1 transcription factors	CEP751, PI103, ZSTK474