

Metastasis: from dissemination to organ-specific colonization

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Abstract | Metastasis to distant organs is an ominous feature of most malignant tumours but the natural history of this process varies in different cancers. The cellular origin, intrinsic properties of the tumour, tissue affinities and circulation patterns determine not only the sites of tumour spread, but also the temporal course and severity of metastasis to vital organs. Striking disparities in the natural progression of different cancers raise important questions about the evolution of metastatic traits, the genetic determinants of these properties and the mechanisms that lead to the selection of metastatic cells.

Infiltration

The entry of cancer cells into distant organs through invasion and extravasation.

Colonization

The outgrowth of metastatic cells that have co-opted a distant organ microenvironment.

Latency

The time between primary tumour diagnosis and clinically detectable metastatic outgrowths.

Intravasation

The entry of tumour cells into the bloodstream.

Extravasation

The exit of tumour cells from capillary beds into the parenchyma of an organ.

Tumour progression towards metastasis is often depicted as a multistage process in which malignant cells spread from the tumour of origin to colonize distant organs^{1–3}. However, these basic steps occur in the context of different organs, emerge at different rates and are clinically managed in different ways depending on the type of cancer. Therefore, a current challenge is to incorporate the heterogeneous biology of this process in current models of metastasis research.

A salient feature of metastasis is the ability of different tumour types to colonize the same or different organ sites^{4,5} (TABLE 1). Although an awareness of this ability has prompted a quest to identify the genes that support metastasis to particular organs^{6–9}, it remains unclear to what extent these genes are used by different tumour types that metastasize to the same organ. Furthermore, some tumours have a more restricted range of target tissues than others. For example, prostate cancer metastasis is largely confined to bone¹⁰ and metastasis by ocular melanoma is almost exclusively confined to the liver¹¹. Another important variable is the temporal course of metastasis. Adenocarcinomas of the breast and lung typically relapse within a similar range of organs, including bone, lung, liver and brain^{12,13}. However, the kinetics of metastasis differ between these two tumour types. Breast cancer recurrences are often detected following years or decades of remission^{14,15}, whereas lung cancers establish distant macrometastases within months of diagnosis^{16,17}. Therefore, the competence of cancer cells to infiltrate distant organs is not always accompanied by the competence to overtly colonize these sites. The temporal gap between organ infiltration and colonization produces a period of metastatic latency. How do disseminated cancer cells develop the ability to colonize the host organ? What enables these disseminated cells to survive as latent

infiltrates until they can acquire this competence? The answers to many questions about organ-specific metastasis and its relationship to the cell of origin and to metastatic latency are unknown, but recent progress provides clues and a conceptual framework to investigate these questions.

Metastasis steps, sites and course

The classical simplification of metastasis into an orderly sequence of basic steps — local invasion, intravasation, survival in the circulation, extravasation and colonization — has helped to rationalize the complex set of biological properties that must be acquired for a particular malignancy to progress towards overt metastatic disease (FIG. 1). These biological events have been described¹⁸, and many genetic and epigenetic events have been identified that contribute to the metastatic path. The list starts with the initiating mutations that generate a tumour by providing unlimited proliferative potential, tolerating cell division defects and an unstable genome, maintaining progenitor-like phenotypes and supporting other cell-autonomous functions that generate oncogenically transformed cells¹⁹. Malignant cells might remain addicted to these tumour-initiating alterations throughout the subsequent stages of metastatic progression. Evidence for this hypothesis is provided by the regression of metastatic lesions in mouse breast cancer models that are dependent on a conditional *ErbB2* (also known as *Neu* or *Her2*) oncogene²⁰ or the improved progression-free survival rates of human patients with ERBB2-positive breast tumours treated with ERBB2 antibody therapy²¹.

Oncogenic transformation, however, is not sufficient for metastatic competence, as shown by the fact that many oncogene-driven mouse models of cancer do not automatically establish distant metastases²² or

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At a glance

- Metastasis progression can be viewed as a stepwise sequence of events, which is mediated by different classes of metastasis genes.
- For each type of cancer, the clinical course of these events occurs with distinct temporal kinetics and in unique organ sites.
- The long latency period of certain tumour types suggests the further evolution or ‘speciation’ of malignant cells in the microenvironments of a particular organ. The acquisition of pro-metastatic functions earlier during primary tumour formation might enable other cancer subtypes to relapse more quickly.
- The organ specificity of metastatic cells is determined by unique infiltrative and colonization functions required after their dissemination from a primary tumour.
- New insights into the importance of latency and organ-specific colonization should be considered in the design of optimized therapeutic strategies.

the observation that some patients have disseminated cancer cells but do not develop clinical metastasis²³. Transformed cells must therefore acquire additional abilities to surmount the natural barriers against metastasis. In addition to forming a locally aggressive tumour, cancer cells must enter the circulation and then exit it to infiltrate distant organs. After infiltrating new tissue, cancer cells will form an aggressive colony if they can survive and then overtake that tissue. Thus, distant organ infiltration and colonization (separated by a variable period of intervening latency) are general steps that primary tumour cells must accomplish to metastasize.

If the stepwise sequence of events that take cancer cells from their site of origin to a distant macro-metastasis is one dimension of metastasis, the kinetics of metastatic progression are a second dimension and the organ sites in which these steps occur define a third one. The barriers to infiltrate each organ and the composition of the microenvironment of each organ are unique (FIG. 2). Therefore, the general steps of metastasis might be the same in all tumour types but metastasis to different organs might require distinct sets of infiltration and colonization functions, which are acquired over variable periods of time.

General classes of metastasis genes

The genes and activities that underlie these general steps of metastasis can be grouped into several classes, which we define as metastasis initiation, metastasis progression and metastasis virulence genes^{24,25} (FIG. 1).

Genes that allow transformed cells to invade the surrounding tissue and attract a supportive stroma also facilitate the dispersion of cancer cells and probably continue to do so after cancer cells infiltrate distant tissues. The genes that determine these activities can be defined as metastasis initiation genes. These genes could promote cell motility, epithelial–mesenchymal transition (EMT), extracellular matrix degradation, bone marrow progenitor mobilization, angiogenesis or evasion of the immune system. For example, EMT is mediated by developmental programmes that are under the control of aberrantly regulated transcription factors, such as *TWIST1*, *SNAI1* and *SNAI2* (also known as *SLUG*)²⁶. Other determinants of invasion are components and modulators of the hepatocyte growth factor (*HGF*)–HGF receptor (*HGFR*) pathway, such as *metadherin* in breast cancer²⁷ and the metastasis-associated in colon cancer 1 (*MACC1*) gene in colorectal carcinoma²⁸. Metastatic growth is also initiated by the suppression of non-coding RNAs, such as *miR-126* and *miR-335* in breast and colorectal carcinomas^{29,30}. The expression of these metastasis initiation genes and their targets predicts a poor prognosis in particular types of cancer.

The infiltration of distant organs by circulating cancer cells also involves specialized activities that are required for cancer cell passage through capillary walls and survival in the newly invaded parenchyma. Malignant cells that have been freshly released must be capable of these activities to successfully infiltrate distant tissues. Accordingly, these capabilities can be provided by genes that are deregulated as cancer cells depart from a primary tumour. These genes could already be prominently expressed in a primary tumour, although they might have a unique role at a distant site. We refer to this class as metastasis progression genes. Unlike the general invasive activities that are conferred by the expression of metastasis initiation genes, metastasis progression genes could have different functions at the primary site and in distant organs. Examples of these genes and the mechanisms for their selection are discussed in BOX 1 (see also FIG. 3). As the structure and composition of capillary walls and the subjacent parenchyma vary in different organs, the functions required for metastatic infiltration, survival and colonization might also differ depending on the target organ. Thus, the expression of genes in primary tumours that facilitate metastasis to specific organs might provide markers that predict organ-specific relapse. There are also genes that confer activities that are essential for the metastatic colonization of a certain organ and for which expression becomes detectable only in cancer cells that metastasize to those tissues. We refer to this class as metastasis virulence genes because their expression accentuates the metastatic proclivity of disseminated cancer cells that have successfully achieved the previous steps of metastasis initiation and progression. For example, osteoclast-mobilizing factors, such as parathyroid hormone-related protein (*PTHrP*) and interleukin 11 (*IL-11*)^{6,9,31}, do not provide an advantage to breast cancer cells in primary tumours but enable them to establish osteolytic metastases in bone. The deregulated expression of metastatic virulence genes could result from stochastic alterations

Table 1 | Typical sites of metastatic relapse for solid tumours

Tumour type	Principal sites of metastasis
Breast	Bone, lungs, liver and brain
Lung adenocarcinoma	Brain, bones, adrenal gland and liver
Skin melanoma	Lungs, brain, skin and liver
Colorectal	Liver and lungs
Pancreatic	Liver and lungs
Prostate	Bones
Sarcoma	Lungs
Uveal melanoma	Liver

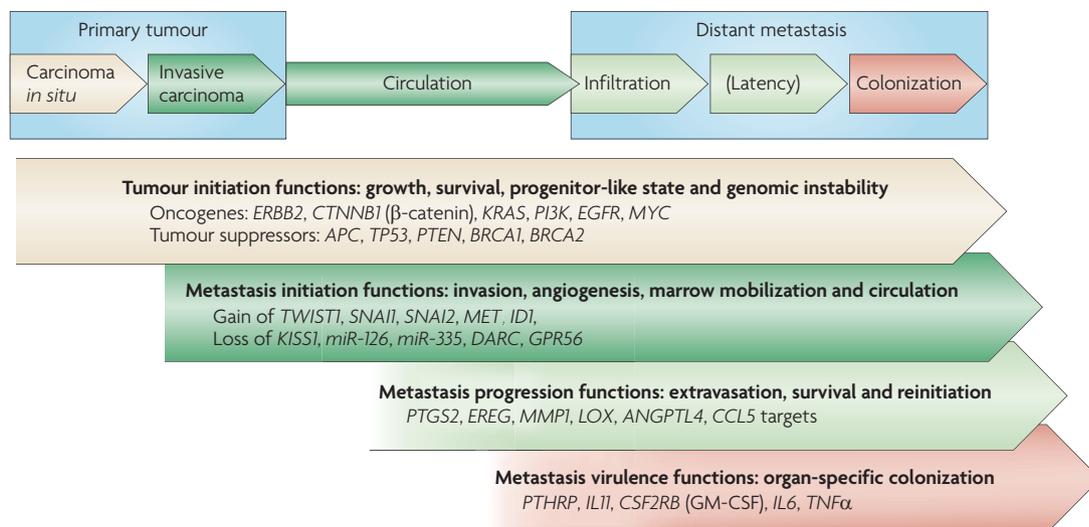


Figure 1 | Basic steps of metastasis and hypothetical classes of metastasis genes. The basic steps of metastasis include the progression of the primary tumour towards invasive carcinoma and dispersion of cancer cells through the lymphatic or blood vessels. Circulating cancer cells that survive could infiltrate distant organs. Infiltrated cells in the new microenvironment might proceed towards overt metastasis with or without an intervening period of latency. These steps are supported by functions of the cancer cells themselves or of the tumour stroma. In addition to the tumour-initiating events that produce an incipient carcinoma (only some examples are listed), metastasis requires functionally distinct classes of genes that provide metastasis initiation, progression and virulence functions. These functions can be acquired through distinct genetic or epigenetic alterations, and might collectively endow circulating cancer cells with the competence to infiltrate, survive in latency and colonize distant organs. ANGPTL4, angiopoietin-like 4; APC, adenomatous polyposis coli; CCL5, C-C chemokine ligand 5; DARC, Duffy antigen chemokine receptor; EGFR, epidermal growth factor receptor; EREG, epiregulin; GM-CSF, granulocyte–macrophage colony stimulating factor; GPR56, G protein-coupled receptor 56; HGF, hepatocyte growth factor; ID1, inhibitor of differentiation 1; IL, interleukin; KISS1, kisspeptin 1; LOX, lysyl oxidase; MMP1, matrix metalloproteinase 1; PTGS2, prostaglandin G/H synthase 2; PTHRP, parathyroid hormone-related protein; TNF α , tumour necrosis factor- α .

in the context of genomic instability, and subsequently their expression could become stabilized as it provides a selective advantage to malignant cells in a particular microenvironment. These genes would not contribute to the expression signatures that are predictive of metastasis in primary tumours.

The temporal course of metastasis

The diverse temporal courses of metastasis in different types of cancer and patient populations are evident from clinical observations. As the kinetics of disease progression and distinct physiological barriers can dictate the latency between the infiltrating and colonizing steps of metastasis, each clinical course has different implications for the organ-selective evolution of metastatic cell populations (FIG. 4). In oestrogen receptor-positive breast cancer, prostate cancer and ocular melanoma, metastasis might become manifest decades after the removal of even a small primary malignancy^{11,32,33}. The absence of immediate clinical relapse implies that these tumour cells are not fully competent to overtake organs immediately after infiltration. A protracted period of latency might ensue during which further malignant evolution of the disseminated cell population, of their microenvironment or of both must occur for colonization to proceed.

In other types of cancer, however, metastasis follows a swift course with rapid expansion in multiple organs that leaves little margin for speciation of the metastatic cell

population. For example, in lung cancers and pancreatic adenocarcinomas, malignant cells might rapidly acquire activities that confer both infiltration and colonization competence, as implied by the short time between primary tumour diagnosis and metastatic relapse in these diseases^{16,34}. In tumours with a rapid course of metastasis, the acquisition of robust metastatic traits in the primary tumour would obviate the need for extensive adaptation on dissemination to distant organs.

Colorectal carcinoma is a defined paradigm of malignant progression and most metastatic traits seem to be acquired during local progression in the primary site. The transition from one stage to the next — from colorectal hyperplasia to adenoma to invasive carcinoma — is characterized by the acquisition of specific genetic alterations over a protracted period of up to three decades³⁵. Colorectal tumours are initiated by the activation of the canonical Wnt pathway, through either mutational inactivation of the tumour suppressor adenomatous polyposis coli (*APC*) or activation of the pathway co-activator β -catenin³⁶. The transition to carcinoma occurs with mutational activation of *KRAS*³⁷, followed by oncogenic activation of the PI3K pathway³⁸, inactivation of *TP53* (REF. 39) and loss of the transforming growth factor- β (TGF β) tumour suppressor pathway⁴⁰. Once a colon tumour invades the underlying colonic wall, metastatic progression can proceed without latency. Colorectal tumours predominantly spread along the mesenteric circulation to the liver in 80% of patients

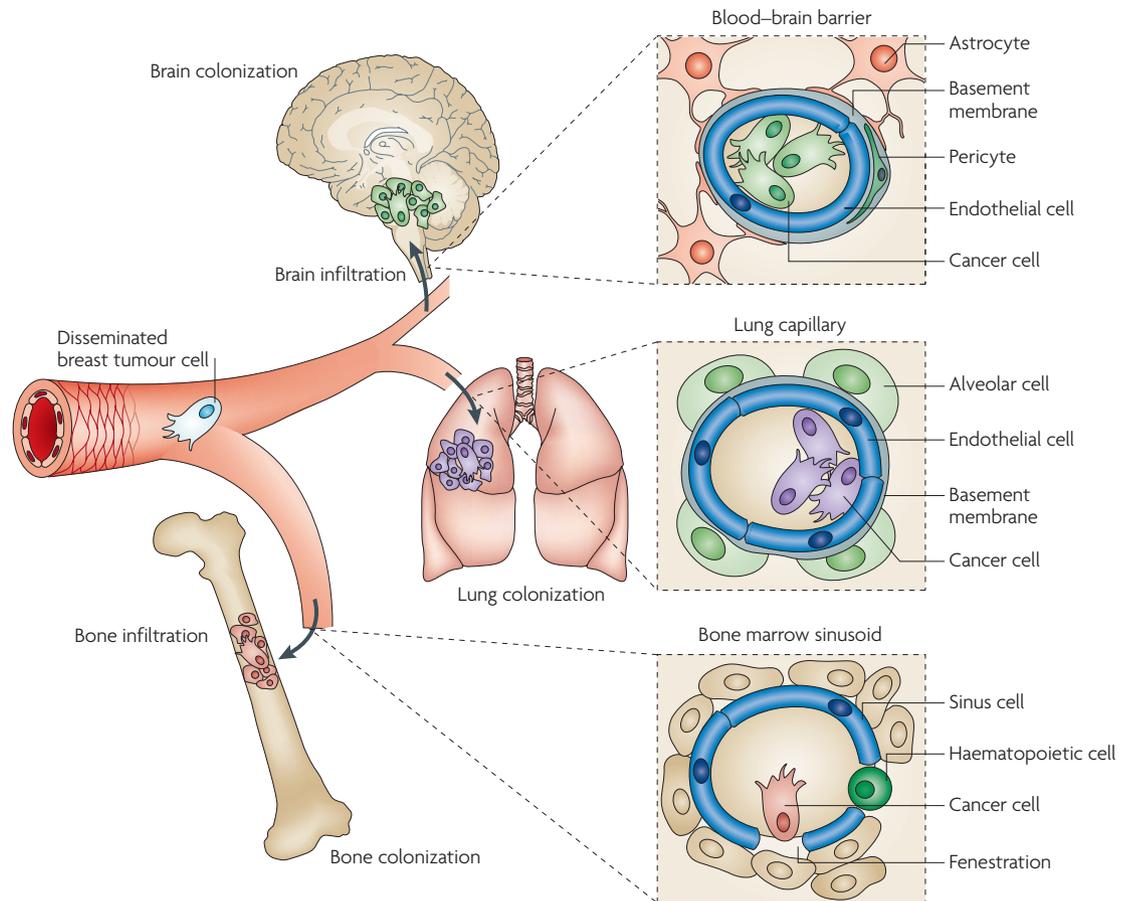


Figure 2 | Organ-specific barriers to metastatic infiltration. The potential barriers to metastasis in different sites are exemplified by the case of breast cancer and the anatomy of capillary walls in different target tissues. Breast cancer cells entering the circulation can infiltrate a distant organ if they carry the necessary functions for extravasation. The fenestrated structure of bone marrow sinusoid capillaries is more permissive to cancer cell infiltration than the contiguous structure of lung capillary walls. Brain capillaries are more difficult to penetrate, owing to the unique nature of the haematoencephalic barrier. Infiltration through these barriers selects for tumour cells that express the necessary extravasation functions. These functions can be provided by genes for which expression in primary tumours independently provides a selective growth advantage (such as vascular remodelling) or by genes for which expression in primary tumours provides no benefit but is a consequence of tumour microenvironment signals.

with recurrent disease¹². It is estimated that most genetic alterations for metastasis are acquired during progression to the invasive carcinoma stage, and few, if any, additional genetic events are required for the formation of distant liver metastases⁴¹. Therefore, colorectal cancers progress slowly to invasive carcinomas but progress rapidly from this stage to the metastatic phase (FIG. 4).

General versus organ-specific infiltration

To enter the circulation and infiltrate distant organs, aggressive cancer cells must invade the surrounding tissues. Various mechanisms that confer invasiveness, such as cellular motility and basement membrane degradation, have been proposed to mediate cancer cell entry into the circulation^{42,43}. Deregulated cytoskeletal modifiers such as *RHOC* can specifically enhance metastatic dissemination⁴⁴. The aberrant expression of developmental transcription factors might trigger EMT, which is associated with cellular plasticity and invasion²⁶.

The capacity to disseminate could be intrinsic to certain pre-malignant cell lineages. It has long been recognized that many normal cell types are involved in complex migratory and invasive behaviours during development and adulthood. The traffic and homing to peripheral tissues of bone marrow-derived progenitors of myeloid, endothelial and mesenchymal lineages have been characterized. Normal epithelial cells are also motile. In the mammary gland, the invasive and migratory mechanisms that underlie the branching morphogenesis of normal epithelial cells also regulate the formation of mammary hyperplasia⁴⁵. A subset of luminal progenitors in early breast carcinomas might use these invasive and migratory mechanisms to disseminate to the lungs⁴⁶. Depending on their cellular origin, epithelial stem cells or progenitors that leave their original niche might have intrinsic invasive capabilities that are independent of malignant transformation. Indeed, a proportion of normal murine pre-malignant mammary cells can infiltrate the lungs when

Box 1 | Mediators of metastasis in the primary tumour

The expression in primary tumours of genes that mediate metastatic activities might seem paradoxical. How could a gene that has a specific function in a distant organ be selected for before primary tumour cells become exposed to the selective pressures of that unique microenvironment? Various answers are suggested by recent progress. The expression of metastasis initiation genes in primary tumours is driven by the need for cell mobility, invasiveness, angiogenesis and immune evasion during the outgrowth of primary tumours, as well as the subsequent outgrowth of distant metastases. However, the prominent expression of metastasis progression genes in primary tumours has a more complex basis. Expression of prostaglandin G/H synthase 2 (PTGS2; also known as COX2), matrix metalloproteinase 1 and the epidermal growth factor receptor ligand epiregulin in breast cancer cells promotes angiogenesis in experimental mammary tumours. When expressed in cancer cells entering the circulation, this set of genes also increases the ability of the disseminated cells to extravasate into the pulmonary parenchyma⁶¹. These metastasis progression genes are prominently expressed in a primary tumour because they support tumour growth through one particular effect, whereas they enhance distant metastasis through another effect.

The boundaries between metastasis initiation and metastasis progression genes are not rigid. Mediators of metastasis that were previously thought to regulate one activity might also confer activities that participate in other processes. For example, the hypoxia-regulated gene lysyl oxidase is predictive of relapse in human breast tumours and was initially found to enhance cancer cell invasion¹⁰⁶. Recent studies suggest that systemic secretion of lysyl oxidase into the lung and liver might facilitate the homing of disseminated cancer cells to these organs through effects on the extracellular matrix that help to recruit CD11b⁺ myeloid cells, forming a pro-metastatic microenvironment¹⁰⁷.

In other situations, the expression of pro-metastatic genes in a primary tumour might be one of many bystander events that do not contribute to primary tumorigenesis. For example, the expression of these genes could be part of a global response to cytokines in the tumour microenvironment (FIG. 3). Bone marrow progenitors¹⁰⁸, endothelial cells¹⁰⁹, macrophages and other myeloid cells^{110,111}, as well as mesenchymal progenitor cells⁶², are stromal components that release paracrine factors in response to malignancy. Although some of these signals are co-opted by the tumour for growth, others are neutral to primary tumour development but might prime cancer cells for distant metastasis. CCL5 is released by bone marrow-derived mesenchymal progenitor cells infiltrating into mammary tumours and stimulates cancer cells to metastasize to the lung without affecting tumorigenesis⁶². Transforming growth factor- β (TGF β) is a prominent cytokine in the tumour microenvironment¹¹². TGF β induces the expression of a large set of genes in breast tumour cells, among which angiopoietin-like 4 (ANGPTL4) disrupts endothelial cell–cell contacts without providing any discernable benefit in mammary tumours. However, cancer cells departing from TGF β -rich primary tumours and expressing ANGPTL4 have an infiltration advantage as they reach the lung capillaries⁶³. Metastasis progression genes in other cancer models await further characterization.

injected into the circulation⁴⁷. Moreover, some human mammary epithelial cell types are more metastatic than others following experimental transformation with defined oncogenes⁴⁸. The potential for dissemination of tumour cells can be further enhanced by signals that induce EMT, which also augment the fraction of progenitor-like cancer cells⁴⁹.

Certain aspects of the vasculature have been proposed to contribute to dissemination. For example, the metastasis suppressor CD82 (also known as KAI1) normally anchors tumour cells to the endothelium through its interaction with the Duffy antigen chemokine receptor (DARC), inducing the senescence of bound epithelial cells⁵⁰. Loss of CD82 therefore facilitates metastatic spread. Tumour cell–platelet interactions could also enable dissemination by forming cell aggregates that protect tumour cells from immune surveillance^{51,52} or by collaborating during extravasation^{53,54}.

Although some general mechanisms of dissemination enable tumour cells to abandon the primary tumour and reach distal organs, more specialized mechanisms might be necessary for the infiltration of specific organs. Four of the most common sites of secondary relapse include the bone marrow, lungs, liver and brain. Infiltration into these organs is influenced in part by circulation patterns. In colorectal carcinoma, the mesenteric circulation from the bowels and the permissiveness of the liver capillary sinusoids are thought to favour liver metastasis^{55,56}. Following blood flow patterns from the liver or from primary tumours on the descending colon, the second most frequent sites of metastasis are the lungs¹². However, in addition to the influence of haematogenous dynamics, colon carcinoma cells preferentially adhere to the liver and lung endothelia, suggesting the existence of specific molecular interactions that favour the retention of tumour cells in these organs⁵⁷. The role of unique endothelial surface molecules as target sites for compatible disseminating cancer cells has been shown in breast cancer cell lines overexpressing the cell adhesion molecule metadherin. In a mouse model, metadherin specifically bound to the pulmonary vasculature and enhanced lung metastasis⁵⁸.

In addition to the possible role of organ-specific endothelial adhesive interactions, we must consider how the structural features of capillary walls in different organs can affect infiltration (FIG. 2). The capillaries in the bone marrow, called sinusoids, are lined with fenestrated endothelia to facilitate the traffic of haematopoietic cells⁵⁹. Therefore, the bone marrow sinusoids are likely to be more permissive to circulating tumour cells. The capillaries of the liver are also fenestrated and readily traversed by tumour cells compared with other organs^{55,56}. By contrast, lung capillaries are lined with endothelial cells that are surrounded by a basement membrane (the fusion of two basal laminae) and adjacent alveolar cells. The basement membrane is an obstacle that circulating tumour cells can bypass only by expressing specific mediators of transendothelial migration^{60–63}. Additional contacts between the tumour cells and an exposed basement membrane might facilitate the infiltration of the target organ, as exemplified by the interactions between $\alpha 3\beta 1$ integrin on breast cancer cells and laminin 5 on lung capillary basement membranes⁶⁴. The blood–brain barrier, with its tight layer of endothelial cells and astrocyte foot processes, represents more of an obstacle⁶⁵. Consequently, the infiltration of circulating cancer cells into the brain parenchyma could require highly specialized functions, many of which remain to be characterized.

Several mediators of pulmonary extravasation have been recently identified and are upregulated in the primary tumours of breast cancer patients that relapse to the lungs^{61,66}. These include epiregulin, prostaglandin G/H synthase 2 (PTGS2; also known as COX2), matrix metalloproteinase 1 (MMP1) and MMP2, which support not only vascular remodelling in primary tumours, but also lung extravasation⁶¹. Another specific mediator of lung extravasation is the cytokine angiopoietin-like 4 (ANGPTL4), which enhances the infiltration of tumour cells into the lungs by inducing the dissociation of endothelial cell–cell junctions⁶³. However, the absence of a robust association

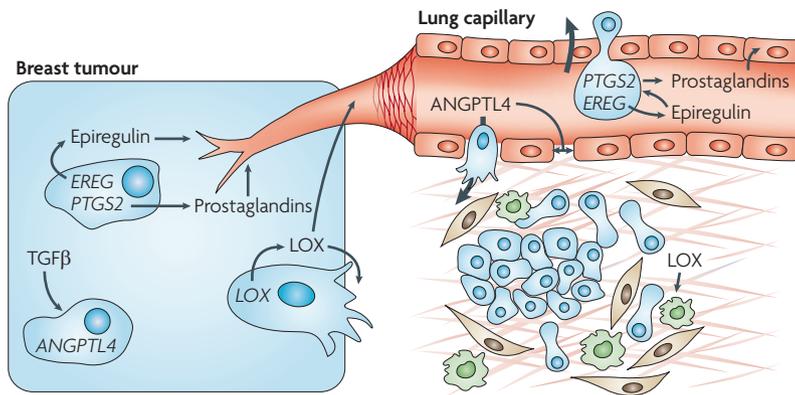


Figure 3 | Metastasis progression genes expressed in the primary tumour. Mediators of metastasis might have dual functions that provide both a local advantage for malignant progression in the primary tumour and a distal advantage for infiltration of a particular organ, such as the lung in a breast cancer patient in this example. The expression of genes such as epiregulin (*EREG*) and prostaglandin G/H synthase 2 (*PTGS2*) promotes capillary assembly from endothelial and smooth muscle cells in mammary tumours. However, these genes also increase the ability of breast cancer cells to pass through endothelial barriers, a function that increases cancer cell extravasation in the lungs. Lysyl oxidase (*LOX*) is induced in primary tumours that respond to hypoxic signals to enhance cancer cell invasion. However, systemic secretion of *LOX* leads to its accumulation in the lung, where it has been suggested to act on extracellular matrix proteins to establish a permissive niche for infiltrating cancer cells. In the case of the cytokine angiopoietin-like 4 (*ANGPTL4*), expression in mammary tumour cells is driven not by a selective growth advantage, but by the action of tumour-derived transforming growth factor- β (*TGF β*), which also stimulates the expression of many other genes. By itself, *ANGPTL4* does not provide any discernable advantage in the primary tumour, but its induction by *TGF β* in departing tumour cells primes these cells for infiltration of the lungs. *ANGPTL4* dissociates vascular endothelial cell–cell junctions, an effect that in lung capillaries increases the infiltration of *ANGPTL4*-secreting cancer cells into the lung parenchyma.

between any primary tumour gene expression event and bone metastasis might reflect the more permissive nature of bone marrow sinusoids and hence less of a requirement for extravasation functions in the departing breast cancer cells that enter the bone marrow^{7,67}.

Metastasis without intervening latency

The duration of metastatic latency and the sites in which it occurs have implications for the development of organ-specific metastasis functions. In some types of cancer, aggressive macrometastases frequently develop soon after cancer cells infiltrate distant organs; examples include lung and pancreatic adenocarcinomas, which are two highly prevalent types of cancer with high relapse and mortality rates following initial diagnosis. The relapse rate is substantial even following the detection of early-stage tumours. For example, the 5-year recurrence-free rate in stage I lung adenocarcinoma patients is 60–70% (REFS 17,68). By contrast, patients diagnosed with stage I breast cancers have a 98% 5-year recurrence-free survival rate⁶⁹, and differences in diagnostic modalities alone do not account for this difference. Patients with limited-stage small-cell lung cancer are even more likely to have metastatic disease at the time of diagnosis⁷⁰. Moreover, lung adenocarcinoma relapses to brain, bone and the contralateral lung, and metastases to these various sites frequently occur concomitantly¹². Malignant skin melanoma can also relapse swiftly,

spreading to cutaneous tissues, lungs, liver, brain and bone. Recurrence in melanoma usually occurs within 2 years of diagnosis, with few relapses after 5 years⁷¹. There are also differences between different tumour subtypes; for example, basal breast cancers classified by gene signatures relapse earlier than luminal breast cancers⁷².

The short latency of metastatic relapse in aggressive diseases implies that potent multi-organ metastatic competence either exists in the pre-malignant cells or is acquired during the early stages of malignant transformation. The early acquisition of robust multi-organ metastatic competence might obviate the need for extensive adaptation by cancer cells to the microenvironment of different affected organs. The determinants of this competence remain unknown and are a topic of intense investigation. The cell and tissue of origin might provide a partial explanation for such rapid metastasis. During melanoma progression, for example, the melanocyte lineage specification programme predisposes to transformation by lineage-determining oncogenes such as microphthalmia-associated transcription factor (*MITE*), a key transcription factor for melanocyte lineage survival⁷³. It has also been proposed that melanocytes retain some embryonic plasticity owing to their neural crest origin. The transcription factor *SNAI2* is required for neural crest cell migration and its expression in melanoma cells drives metastasis to multiple organ sites in mice⁷⁴.

The influence of the epigenetic state of progenitor cells on their metastatic competence might extend to other cancers. Indeed, different subsets of solid tumours that express transcriptional modules that are unique to embryonic stem cells have a higher probability of general recurrence⁷⁵. The embryonic-like plasticity of aggressive cancer cells might reflect or phenocopy the plasticity that is inherent to stem and early progenitor cells, which is maintained in part by global epigenetic regulators. Some of these regulators have been linked to metastasis, including polycomb chromatin remodelling complexes^{76,77} and microRNAs^{30,78–80}. The selection for activated developmental pathways might also enhance metastatic competence to multiple organs by enforcing this plasticity and providing strong invasive and adaptation functions to cancer cells. Identifying the mechanisms that promote metastatic progression without latent speciation might indicate crucial therapeutic targets for early intervention.

Metastatic latency and its host sites

The counterpoint to tumour types that rapidly colonize distant organs with a short disease-free interval on initial diagnosis are tumours that can efficiently infiltrate distant organs at early stages but are unable to promptly grow as macrometastases. In breast cancer, disseminated tumour cells (DTCs) enter a state of metastatic latency, which is defined as the time between primary tumour diagnosis and clinically detectable metastatic relapse^{15,81}. Malignant cells from breast tumours that disseminate early can reside as single cells or as micrometastatic clusters, as shown in studies of bone marrow samples from patients without overt metastatic disease^{14,82,83}. These DTCs either lack the ability to colonize or are prevented

Basal breast cancer
A more aggressive subtype of breast cancer with characteristics of mammary basal cells, and that typically lacks oestrogen and progesterone receptors.

Luminal breast cancer
A subtype of breast cancer with characteristics of cells that originate from the normal lumen or ducts of the mammary gland.

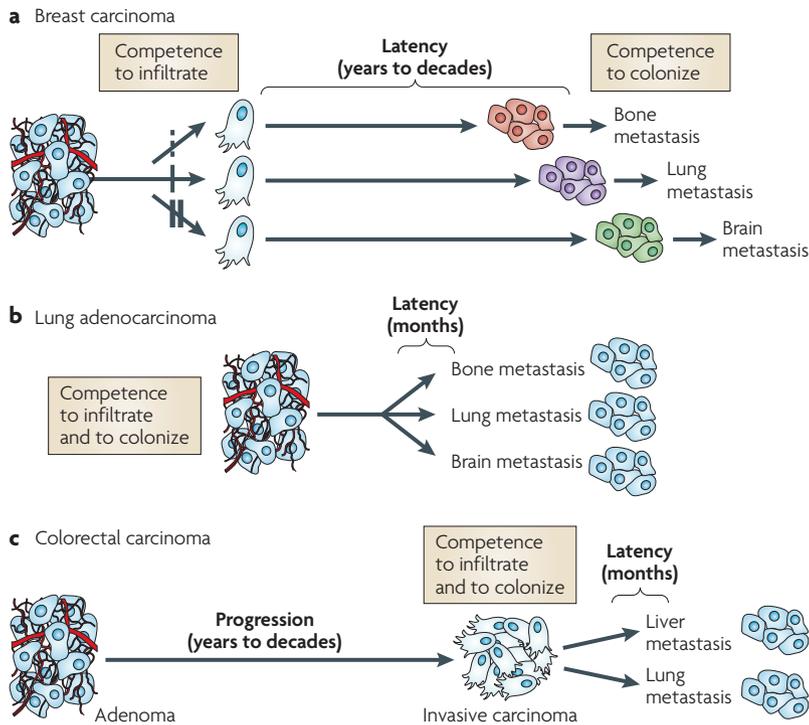


Figure 4 | The temporal course of metastasis. A model depicting the mode of metastatic progression as a function of space and time. The course of metastasis can vary according to the tumour type. **a** | In oestrogen receptor-positive breast tumours, cancer cells can be competent to disperse and infiltrate distant organs at early stages but they frequently enter a prolonged period of latency. During this period, disseminated cancer cells can remain dormant or enter a proliferative state that is counterbalanced by cell death. Through unknown mechanisms, a subset of these latent tumour cells (or their microenvironment) can accumulate the full set of functions that are required for overt colonization. In this model, disseminated breast cancer cells complete their evolution into metastatic entities under selection in a particular host microenvironment, producing organ-specific metastases. **b** | Lung adenocarcinoma cells also target the brain, bone and contralateral lung but do so without a long intervening lag between infiltration and colonization. This course of metastasis implies the existence of mechanisms that render lung adenocarcinoma cells competent for infiltration and colonization of multiple organs. **c** | Colorectal adenomas can take decades to develop into locally invasive carcinomas but, once this stage is reached, dissemination and colonization of the liver and, less frequently, of the lungs rapidly ensue. Therefore, the different courses of metastasis in these types of cancer imply different mechanisms for the acquisition of infiltration, survival and colonization functions.

from displaying colonization by the environment. As a result, DTCs can enter a state of proliferative dormancy by exiting the proliferative cycle for an indefinite period. Alternatively, DTCs grow indolently as micrometastatic colonies owing to a rate of cell death that counterbalances the rate of proliferation, which gives rise to ‘tumour mass dormancy’. The two general forms of latency are not mutually exclusive and could coexist in the DTC population of a particular cancer patient (FIG. 5).

Both the organ microenvironment and the oncogenic background might play important parts in forcing metastatic latency. In a polyoma middle T antigen (PyMT) mouse model, tumour cells lacking $\beta 1$ integrins fail to sense fibronectin as an environmental cue, resulting in growth arrest⁸⁴. Stress signals stemming from

the foreign microenvironment have been proposed to induce dormancy by modulating the ratio of Erk and p38 MAPKs in DTCs^{85,86}. Interestingly, DTCs obtained from transgenic tumour models and transplanted into the marrow of wild-type recipients can expand in the recipient marrow, suggesting that the dormant state can be rapidly discontinued by changes in the microenvironment⁸³. The expression of active metastasis suppressor genes could also contribute to metastatic latency, as exemplified by kisspeptin 1 (*KISS1*), which prevents metastatic cells from reinitiating growth on infiltration of distant organs⁸⁷. Another metastasis suppressor is the G protein-coupled receptor GPR56, which interacts with tissue transglutaminase from the extracellular matrix. Loss of GPR56 expression in metastatic melanoma cells promotes tumour outgrowth⁸⁸. Furthermore, host polymorphisms can modulate the efficiency of tumour metastases, as exemplified by the *Sipa1* polymorphism that has been described in mice⁸⁹. The failure of micrometastatic lesions to trigger the angiogenic switch owing to local anti-angiogenic factors such as thrombospondin has also been associated with dormant metastasis^{90–92}.

Although most breast cancer cells that enter the circulation and infiltrate distant organs die owing to restrictive forces in the host microenvironment⁹³, other factors could provide unique advantages to infiltrating cancer cells that are equipped to exploit survival signals. The bone marrow is a permissive niche for the traffic and residence of haematopoietic stem cells⁵⁹ and seems to be a protective environment for disseminated tumour cells in patients undergoing chemotherapy⁹⁴. These observations suggest that the bone marrow might provide survival signals that sustain the viability of DTCs. C-X-C chemokine receptor 4 (*CXCR4*) is the receptor for the cell survival chemokine stromal cell-derived factor 1 (*SDF1*; also known as CXCL12), which is produced by mesenchymal cells in the bone marrow. Notably, *CXCR4* expression in breast cancer cells is a marker and mediator of bone metastasis in breast cancer^{9,95}. Therefore, *SDF1* and *CXCR4* are candidate mediators of latent DTC survival in the bone marrow (FIG. 5).

To be compatible with eventual macrometastatic outgrowth, latent DTCs require not only the ability to survive during latency, but also the capacity to reinitiate a tumour when conditions are favourable. The development of macrometastases in patients who have isolated DTCs is a manifestation of the ‘tumour-propagating phenotype’ — also referred to as the ‘cancer stem cell phenotype’ — which DTCs require for the eventual reinitiation of aggressive tumour growth. The expression of inhibitor of differentiation 1 (*ID1*) and *ID3* supports the ability of human breast cancer cells to bypass senescence and reinitiate growth on extravasation into the lungs of mice. Furthermore, the expression of *ID1* and *ID3* in cell clusters of basal or triple-negative subtype breast tumours is associated with lung metastasis^{96,97}. These examples suggest that the ability to reinitiate growth at the secondary site can be stochastic owing to newly established interactions between the tumour cell and the target microenvironment or can be already encoded

Dormancy

A state of cellular quiescence in the G₀ phase of the cell cycle. When referring to a tumour cell mass, dormancy describes a balanced state of proliferation and apoptosis.

Angiogenic switch

The transition between a non-angiogenic state of the tumour cell mass and a neovascularized state that enables tumour oxygenation and growth.

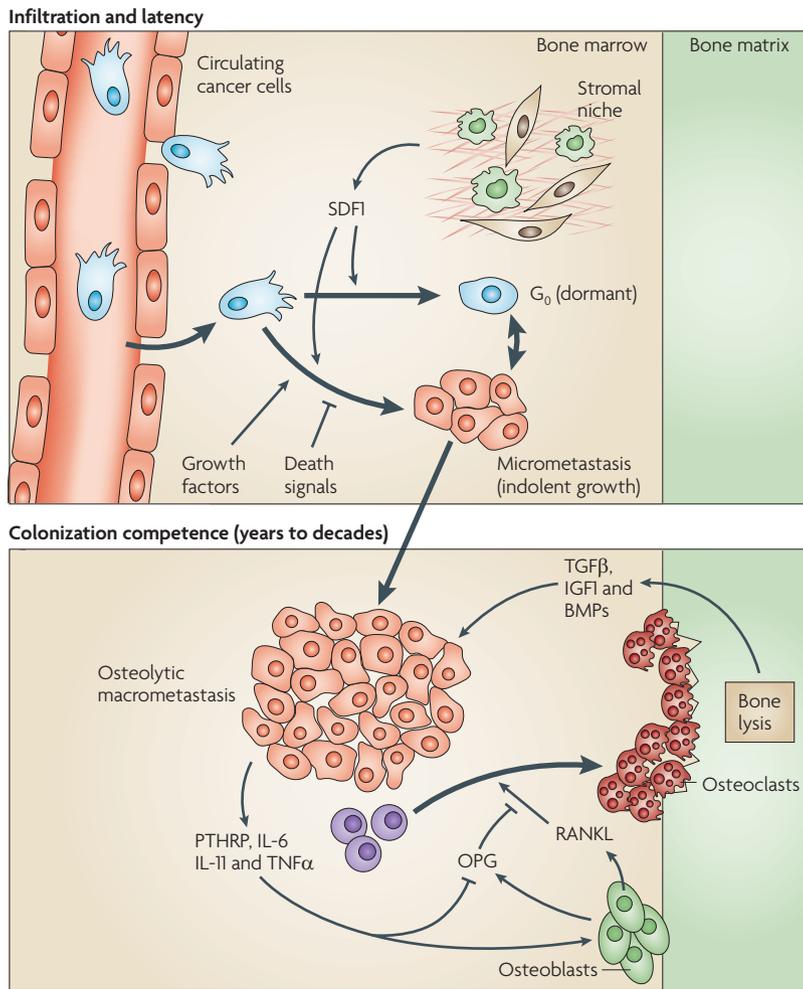


Figure 5 | Metastatic speciation of latent disseminated tumour cells. A model for the survival and emergence of latent disseminated tumour cells (DTCs) in the bone marrow. Once circulating cancer cells have successfully infiltrated the bone microenvironment, they might encounter a balance of growth-promoting and death signals in the newly infiltrated stroma. Following infiltration, this balance of signals can be detrimental to overt metastatic colonization, forcing DTCs into a state of growth arrest or indolent micrometastatic growth. Cells equipped with the appropriate genetic or epigenetic makeup can co-opt more specific cues for survival. For example, cancer cells expressing C-X-C chemokine receptor 4 (CXCR4) might respond to the pro-survival chemokine stromal cell-derived factor 1 (SDF1; also known as CXCL12), which is abundantly produced by resident mesenchymal cells in the bone marrow. Successful emergence from latency is the result of further evolution of surviving DTCs into metastatic populations that have acquired the competence for colonization over a protracted period of time. In the case of breast cancer, bone marrow DTCs that gain the ability to secrete parathyroid hormone-related protein (PTHRP), tumour necrosis factor- α (TNF α), interleukin 6 (IL-6) and/or IL-11 stimulate the release of receptor activator of nuclear factor- κ B ligand (RANKL) from osteoblasts and suppress the release of the RANKL antagonist osteoprotegerin (OPG). RANKL in turn stimulates the development of osteoclasts from myeloid precursors. Activated osteoclasts degrade the bone matrix, releasing cytokines that are normally stored in the bone matrix: transforming growth factor- β (TGF β), bone morphogenetic proteins (BMPs) and insulin-like growth factors (IGFs). These factors in turn can act on the cancer cells and perpetuate a cycle of macrometastasis outgrowth.

in the arriving tumour cell. Identifying the balance of signals that affect DTC turnover and the properties required for these cells to maintain a viable state despite latency should provide valuable clues for therapeutic intervention against minimal residual disease.

Organ-selective metastatic speciation

In malignancies of the breast and prostate, in which relapses occur after a prolonged latency period, the acquisition of competency for colonization is likely to occur during the residence of DTCs in a particular organ microenvironment. The bone marrow, lung, brain and liver parenchyma impose different selective pressures for the establishment of metastatic colonies. Therefore, the eventual colonization of these organs by temporarily latent DTCs could involve the acquisition of specific functions. This process would be predicted to yield organ-specific metastatic cells. Indeed, for a patient with breast cancer, tumour recurrence frequently occurs in one particular organ before it occurs in others. Strikingly, in prostate cancer, bone metastasis is frequently the only site of distant relapse, implying that metastatic prostate cancer cells are not competent to aggressively colonize other organs.

Genetic or epigenetic fluctuation of a DTC population, systemic or local changes in the microenvironment, or a combination of these factors might eventually endow surviving DTCs with full competence for aggressive colonization. Under the selective pressure of the host microenvironment, these events can produce different metastatic cells that are specifically adapted to grow in one particular organ, yielding different 'species' of metastasis in different organs of the same patient. Metastatic cells that are released from distant organs in patients with advanced metastatic disease can commingle in the circulation and other fluids, providing a demographic cross section of the extant species of metastasis in that patient. Indeed, malignant cells and cell lines isolated from the pleural fluid of patients with breast cancer produce subpopulations with distinct organ-specific tropisms when inoculated in mice⁷. Notably, these organ-specific metastatic phenotypes are stable *ex vivo*, suggesting that they evolved through the accumulation of genetic or epigenetic alterations that became fixed in the metastatic population. According to this hypothesis, metastatic speciation results from the protracted evolution of latent DTCs towards full metastatic competence at secondary sites (BOX 2).

Organ-specific colonization functions have been well documented in bone metastasis. The ability of breast cancer cells to form typical osteolytic metastases requires the production of osteoclast-activating factors, such as PTHRP, IL-11, IL-6, tumour necrosis factor- α (TNF α) and granulocyte-macrophage colony stimulating factor (GM-CSF)^{6,9,31,98}. PTHRP, IL-6, IL-11 and TNF α act on osteoclasts to promote the secretion of receptor activator of nuclear factor- κ B ligand (RANKL), which induces osteoclast formation. GM-CSF directly promotes osteoclastogenesis. Expression of these secreted factors would be unlikely to provide a selective advantage in another metastatic site or in the primary tumour, yet they are essential for the development of osteolytic lesions (FIG. 5). The identity of specific molecular mediators of colonization in other organ microenvironments, such as the brain or the liver, remains unknown. However, the range of unique cell types that comprise the brain parenchyma and their anatomical organization⁶⁵ raise the possibility that brain metastasis involves an active crosstalk between

Box 2 | Metastatic speciation from disseminated tumour cells

The presence of disseminated tumour cells (DTCs) in patients whose primary tumours have been removed correlates with metastatic relapse, suggesting that these cells are a source of future recurrence¹¹³. DTCs have been detected primarily in the bone marrow but also in the peripheral blood and lymph nodes. The lack of specific markers and the difficulty of isolating DTCs from other organs preclude us from knowing whether they widely disseminate or the bone marrow preferentially acts as an initial reservoir of DTCs^{114,115}. If the bone marrow acts as such a reservoir, DTCs could evolve from indolent disease into a fully fledged local bone metastasis or, alternatively, evolve until they are competent to seed secondary organs, such as the lung and the brain, in which further organ-specific evolution might occur.

Irrespective of the distant organ location, DTCs will encounter different selective pressures from those at the primary site. DTCs might be unable to survive owing to their failure to establish productive interactions with this newly infiltrated environment. Alternatively, DTCs can use existing components of their cellular machinery to derive an advantage from newly encountered survival cues. Depending on how DTCs respond to local signals, a population of DTCs can continue growing or enter a phase of balanced proliferation and apoptosis until sufficient random genetic and epigenetic variation accumulates for metastatic expansion of clones that are optimally adapted to the host microenvironment. We call this process *metastatic speciation*.

DTCs obtained from the bone marrow, lymph nodes and blood of individual cancer patients exist in a diverse genomic state¹¹⁶. Using single-cell comparative genomic hybridization, DTCs isolated from the bone marrow after primary tumour resection were found to have fewer aberrations than the primary tumour they were derived from. The early dissemination of breast cancer cells and their genetic divergence imply that metastatic lesions and the original tumour could evolve independently^{14,117}. However, it remains unclear whether metastatic outgrowth preferentially occurs from these earliest latent DTCs or initiates from a later seeding of cancer cells that had already become more aggressive in the context of the expanding primary tumour. Indeed, several studies show that overt metastases and most aggressive primary tumours share similar gene expression patterns, implying that at least some metastatic traits are common between metastases and their primary tumour of origin at some stage^{118,119}. Progress in understanding the origin and fate of DTCs, and how they successfully produce secondary tumours, will offer new insights into this intriguing process.

cancer and stromal cells. Consistent with this hypothesis, strong gliosis has been observed in clinical samples of human metastasis, and *in vitro* evidence suggests that glial cells support the growth of metastatic cells⁹⁹.

Influence of therapy on metastatic course

As an end-stage malignant disease, metastatic relapse is often associated with resistance to therapy. Relapse following systemic treatments might be due to cell-intrinsic mechanisms such as genetic alterations that confer drug resistance following a period of therapeutic response. Lung adenocarcinomas with epidermal growth factor receptor (EGFR) mutations respond to EGFR kinase inhibitors but frequently relapse owing to secondary EGFR mutations that confer resistance¹⁰⁰. Certain mechanisms of drug resistance might simultaneously render the tumour more competent for metastasis. For example, a subset of EGFR-mutant lung adenocarcinomas becomes insensitive to the EGFR kinase inhibitors gefitinib and erlotinib owing to the amplification of *MET*, which encodes the tyrosine kinase receptor HGFR. HGFR activation can heterologously increase EGFR signalling, thus promoting the survival of tumours that are addicted to this pathway^{101,102}. However, HGF has a direct role in developmental and pathogenic cell migration^{101,102}. Moreover, *MET* expression is also regulated by metadherin, which

confers resistance to chemotherapy²⁷ and mediates lung metastasis⁵⁸. Consequently, resistance to therapy is coupled with the potential acquisition of pro-metastatic functions in tumours in which the HGF–HGFR pathway is activated.

Alternatively, therapies can indirectly influence the course and pattern of metastasis by delaying systemic disease and favouring the emergence of recurrences in specific organs; this is exemplified by the rising incidence of brain metastasis in ERBB2-positive breast cancer patients treated with the ERBB2 antibody trastuzumab (Herceptin)^{65,103}. The causes of preferential relapse in the brain following systemic therapy are intriguing and have been attributed to the ‘sanctuary’ nature of the brain parenchyma. Cancer cells growing in the central nervous system could be shielded by the blood–brain barrier from drug delivery or protected by survival signals from the host microenvironment¹⁰⁴. The partial effectiveness of adjuvant intervention might increase the incidence of more latent brain metastases, in which DTCs are forced to adapt and acquire specific genetic alterations that favour macrometastatic outgrowth in the central nervous system.

Other specific organ niches could similarly provide microenvironments protective against therapeutic intervention. It has been suggested that DTCs remain quiescent in the bone marrow, providing an explanation for the failure of cytotoxic therapies to treat patients with breast cancer, of whom 15–20% can have residual tumour cells after completing adjuvant cytotoxic and endocrine therapy^{93,105}. In this regard, the identification of tissue-specific prognostic signatures might provide more tailored clinical options. Although these examples show how therapies might select for specific metastatic traits, aggressive metastatic cells could also emerge independently of intervention and be intrinsically resistant to subsequent treatment. This might be particularly relevant in rapidly progressing tumour types, such as lung cancer and melanoma, for which there are few effective treatment modalities other than surgical intervention.

Perspectives

As clinical oncology progresses towards personalized cancer medicine, the need to understand the biology of metastasis becomes increasingly acute. In the past few years, we have witnessed an invigoration of this field, accompanied by technological developments that are enhancing our understanding of how metastasis develops and how it might be amenable to therapy. Three important needs could be addressed at this point. The first is the incorporation of clinical knowledge of the steps, sites and temporal course of metastasis into experimental models of disease. The second is the dissection of metastasis into clinically relevant cellular and molecular components that drive this process in organ-specific patterns. The third is the translation of this information into a better classification of tumours on the basis of molecular markers of metastatic potential and of therapeutic intervention against latent and active metastatic disease. We are optimistic that progress will be made towards these goals in the coming years.

Tumour-propagating phenotype

The ability of the infiltrated tumour cells to reinitiate growth at the secondary site. This is referred to by some investigators as the ‘cancer stem cell phenotype’.

Metastatic speciation

An evolutionary process by which new metastatic populations arise, owing to the various selective pressures that act on the heterogeneous cancer cells escaping the primary tumour.

Gliosis

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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
 APC | KRAS | ID1 | ID3 | MACC1 | MET | TP53
 UniProtKB: <http://www.uniprot.org>
 β -catenin | ANGPTL4 | CD82 | CXCR4 | DARC | EGFR | epiregulin | ERBB2 | GM-CSF | HGF | HGFER | IL-11 | KISS1 | metadherin | MITE | MMP1 | MMP2 | PTGS2 | PTHRP | RANKL | RHOC | SDE1 | SNAI1 | SNAI2 | TNF α | TWIST1

FURTHER INFORMATION

Joan Massagué's homepage: <http://www.mskcc.org/mskcc/html/10614.cfm>

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000 Metastasis: from dissemination to organ-specific colonization

Don X. Nguyen, Paula D. Bos and Joan Massagué

The natural history of metastasis — which appears to be cancer-type specific — varies by target organ, latency and severity. This Review discusses how organ speciation and the competence to colonize might develop.