

Metastatic colonization by circulating tumour cells

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Metastasis is the main cause of death in people with cancer. To colonize distant organs, circulating tumour cells must overcome many obstacles through mechanisms that we are only now starting to understand. These include infiltrating distant tissue, evading immune defences, adapting to supportive niches, surviving as latent tumour-initiating seeds and eventually breaking out to replace the host tissue. They make metastasis a highly inefficient process. However, once metastases have been established, current treatments frequently fail to provide durable responses. An improved understanding of the mechanistic determinants of such colonization is needed to better prevent and treat metastatic cancer.

Malignant tumours start early on the road to metastasis. Cancer cells that are invasive and motile can enter the circulatory system long before a tumour is diagnosed. Most of these cells will perish, but a small proportion manages to infiltrate distant organs and survives as disseminated seeds for eventual relapse. Thus, at diagnosis, a primary tumour might have already seeded distant organs with thousands of cancer cells. These cells will face many obstacles before they can take over their host organ and form clinically relevant lesions. Indeed, organ colonization is the most complex and rate-limiting phase of metastasis.

Research on metastatic colonization has been hindered by the complexity of the biology and a lack of adequate experimental models. However, the development of patient-derived and genetically engineered mouse models of metastasis, enhanced imaging technologies and advanced genomic sequencing, including the ability to analyse single cells, together with improved access to clinically annotated tissue samples, has brought fresh insight to our understanding of the molecular mechanisms that allow circulating tumour cells (CTCs) to invade distant organs, settle in supportive niches and eventually overtake their host tissue (Fig. 1). This has allowed us to better conceptualize the metastatic process as a whole and provides a basis for superior treatments. Such advances show that the elucidation of metastatic colonization is a tractable problem with clinical benefits. In this Review, we highlight current concepts and open questions at the forefront of this field. Each aspect of metastatic colonization that we explore is an individually rich area of research, and we cite specialized articles that cover them in depth.

The inefficiency of metastatic colonization

Even small tumours can shed millions of cancer cells. However, many people who have recovered from cancer never experience relapse or do so only after a long period of latency without clinically manifest disease. The number of CTCs — cancer cells that are found in blood from patients — far exceeds the number of overt metastatic lesions that develop¹. Cancer cells that survive after infiltrating distant organs, known as disseminated tumour cells (DTCs), can be present in the bone marrow of people with cancer for years, yet only about half of these patients develop overt metastasis². These clinical observations suggest that metastatic colonization is a very inefficient process in which most cells die and only a minority of those that survive form macrometastases.

Data from experimental mouse models are in line with the clinical evidence. For example, intravenously injected cancer cells that reach the

lungs die in large numbers within 2 days³, as do arterially injected cancer cells that lodge in the brain, liver or bone marrow⁴. And populations of cells that are enriched for highly metastatic cells experience extensive attrition after infiltration of distant organs⁵. The vast majority of melanoma cells that are injected in the portal vein fail to form micrometastases in the liver, and only 0.02% form macrometastases^{6,7}. Similarly, most cancer cells that infiltrate the brain will die^{8–10}. Such inefficiencies cannot be attributed simply to a scarcity of cancer stem cells with metastasis-initiating potential: the majority of breast cancer stem cells that reach the lungs undergo apoptosis¹¹ and colorectal cancer stem cells are cleared quickly after infiltrating the liver parenchyma¹². Observations such as these, in mouse models and in the clinic, imply that the factors that influence the survival and tumour-initiating activity of DTCs are important determinants of metastasis.

Organ infiltration

The early steps in the metastatic cascade, which include the invasion and migration of cancer cells into tissues and the circulatory system, have been extensively studied^{13,14}. Cytoskeletal rearrangements within cancer cells¹⁵, combined with adhesive interactions between cells and the secretion of extracellular-matrix-degrading metalloproteinases (MMPs) and cathepsins^{16,17}, drive their invasion and migration through the stroma, a network of supportive, connective tissue cells. Cancer cells can migrate as single cells boring a path through the extracellular matrix¹⁸, move along collagen fibres¹⁹ or migrate collectively as ensembles that forge ahead of the tumour invasion front²⁰. In prostate cancer, invasion along nerve fibres provides an additional route for dissemination²¹. In response to transforming growth factor- β (TGF- β) and other cell-signalling proteins that are released by stromal cells, carcinoma cells can undergo epithelial-to-mesenchymal transition (EMT) — a reversible phenotypic change in which cells lose intercellular adhesion and epithelial polarization and gain motility and invasiveness²². EMT has an important role in gastrulation and other morphogenic events that occur during development. In carcinoma cells, EMT can promote cell entry into the vasculature, known as intravasation, and support the induction of a stem-cell phenotype, whereas a reversal of this state after extravasation — in which cancer cells exit capillaries to enter organs — can facilitate colonization²³. However, studies in models of breast and pancreatic cancer suggest that EMT could be dispensable for the establishment of metastasis, despite it contributing to the aggressiveness of cancer cells by increasing their

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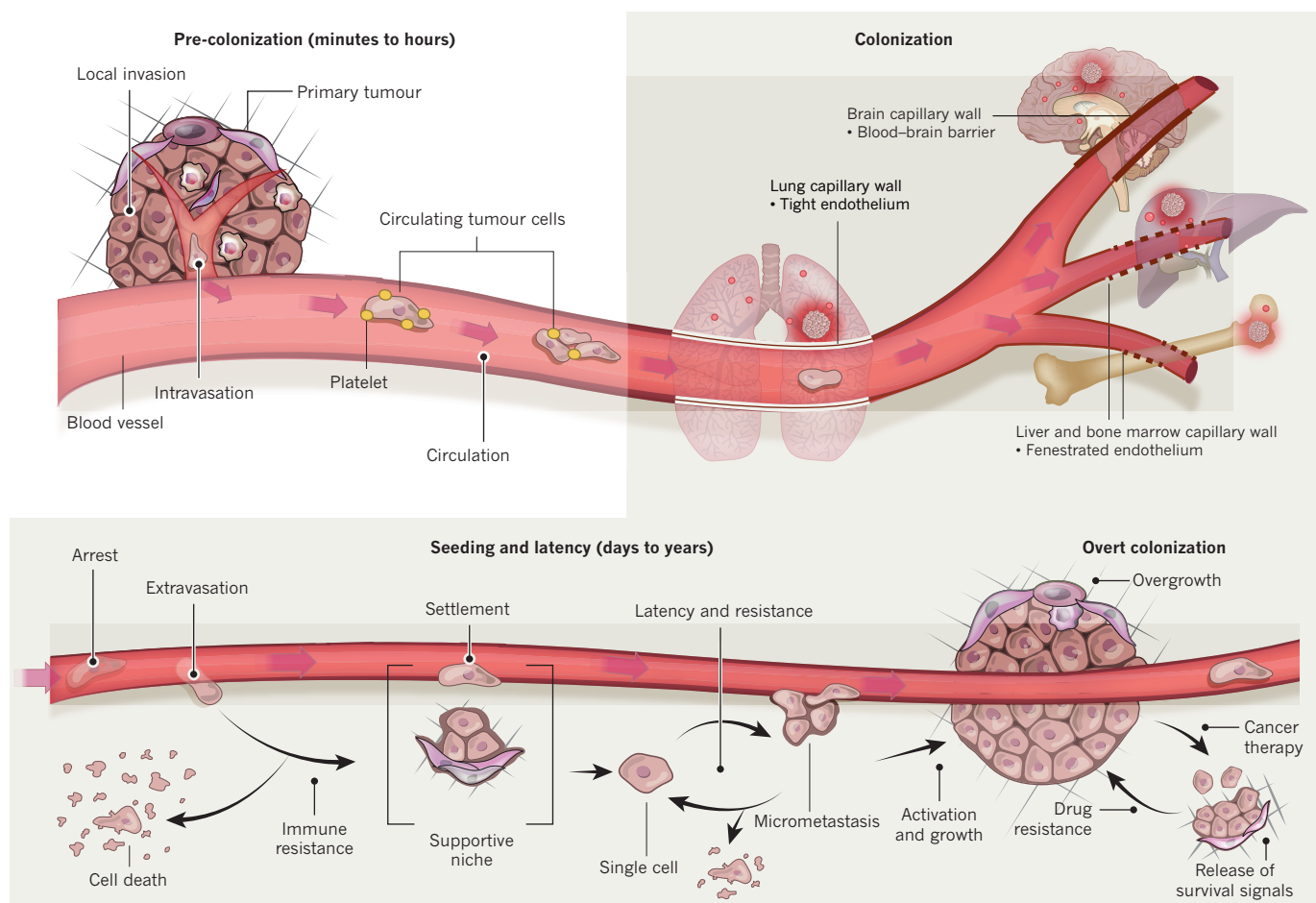


Figure 1 | Metastatic colonization. Metastasis proceeds through multiple steps and restrictive bottlenecks. The pre-colonization phase of metastasis comprises a series of events that occur on a timescale of minutes to hours. Local invasion of the primary tumour by cancer cells is followed by their intravasation into the tumour vasculature. The cancer cells then enter the circulatory system as single cells or clusters that are coated with platelets. Circulatory patterns, which move blood through the lungs and then on to other organs, and the differing structure of the capillary walls in each organ influence the dissemination of CTCs. On their arrest in capillaries at distant sites, the cancer cells extravasate into the parenchyma of target organs to commence colonization. Colonization can be parsed into many steps that occur on a timescale of years. After extravasation, colonizing cancer cells must develop resistance to immunity and other host-tissue defences to survive. Settlement in

chemoresistance^{24,25}. Therefore, the contribution of EMT to metastasis might be more nuanced than thought previously.

Cancer cells can leave tumours as single cells or in clusters (Fig. 1). There is growing evidence to indicate that distinct cancer-cell clones show cooperative behaviour, which promotes their mutual survival and metastatic ability^{26–29}. Polyclonal metastatic seeding, for example, has been documented in patients with prostate cancer³⁰, and in experimental models, polyclonal clusters of CTCs establish metastases more efficiently than single cells³¹.

In the bloodstream, cancer cells are exposed to considerable shear forces, the innate immune system and oxidative stress. To protect themselves during transit, they associate with platelets³² and undergo reversible metabolic changes that increase their ability to withstand oxidative stress³³. For instance, melanoma cells show an increased dependence on nicotinamide adenine dinucleotide phosphate (NADPH)-generating enzymes in the folate pathway, and inhibition of this pathway reduces overt metastasis³⁴.

CTCs become mechanically entrapped in capillaries before they exit the blood and move into tissue. This is considered to be the main mechanism

supportive niches enables them to survive and retain their stem-like tumour-initiating capacity. The cancer cells then enter a latent state as single cells or micrometastases. During latency, which can last from months to decades, disseminated cells must achieve long-term survival. They might also acquire traits that are required to overtake host tissue. When the cancer cells break out of latency, they reinitiate overt outgrowth and overtake the local tissue microenvironment. Therapeutic treatment can partially eliminate clinically manifest metastases. However, under therapy-induced stress, cancer cells and non-neoplastic stromal cells mobilize survival signals that nurture the residual disease until minority drug-resistant clones emerge to lead the outgrowth of a drug-resistant tumour. Different host-tissue microenvironments select for cancer cells with distinct metastatic traits, which gives rise to organ-specific populations of metastatic cells.

for cancer-cell arrest. The first capillary bed that a CTC encounters is determined by patterns of blood circulation in the body. In most organs, the venous circulation leads to the right ventricle of the heart and on to the lungs, although the venous circulation from the gut drains into the liver. The resulting retention of CTCs in the lungs or liver, respectively, contributes to the high incidence of metastasis in these organs³⁵. However, some CTCs bypass these initial filters, perhaps through larger arteriovenous shunts, to reach other organs through the arterial circulation.

CTCs that lodge in the microvasculature can initiate growth within the lumen to form an embolus that eventually ruptures the vessel³⁶ or extravasate by breaching vascular walls^{5,6}. The composition of these walls differs between organs and is another factor that influences where cancer cells extravasate (Fig. 1). The capillaries in the liver and bone marrow, called sinusoids, are lined with fenestrated endothelial cells and a discontinuous basal lamina³⁷ — gaps that might facilitate the extravasation of CTCs and contribute to the high incidence of liver and bone metastasis^{13,38}. In contrast, the endothelium of lung capillaries has tight junctions and a basement membrane, and the capillary walls of the brain are reinforced by pericytes and the processes of astrocytes, which together constitute

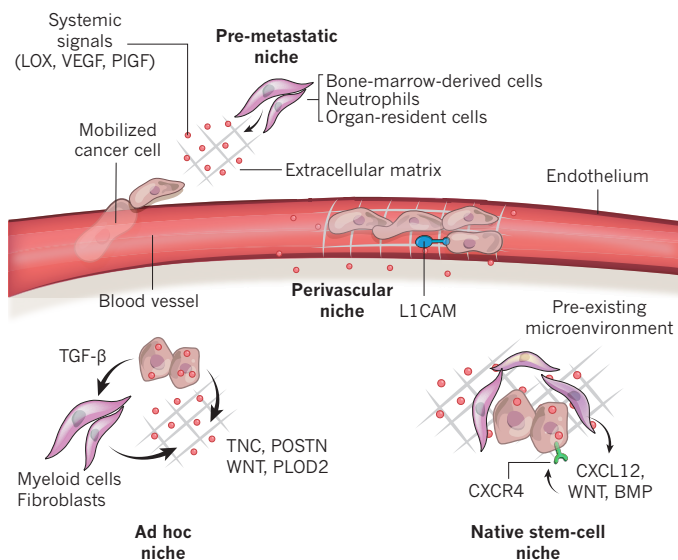


Figure 2 | Metastatic niches. Cancer cells that infiltrate distant tissues survive and retain their stem-cell potential by locating themselves in supportive niches, which are akin to the niches that support normal adult stem cells. A number of different niches have been proposed. Pre-metastatic niches form before the arrival of cancer cells by systemic signals from the primary tumour that recruit supportive stromal cells. Perivascular niches support cancer cells that spread over the capillary basement membrane after extravasation. The cancer cells remain close to cells of the endothelium and their paracrine factors. Ad hoc niches are established by the secretory products of cancer cells and act in an autocrine manner or recruit stromal components as sources of supportive signals. Native stem-cell niches of the host tissue are often invaded by the infiltrating cancer cells, which allows the cells to occupy directly a supportive microenvironment. The location or composition of these niches can overlap. For example, a native stem-cell niche might be in a perivascular site, or pre-metastatic signals might combine with those released by the cancer cell at an ad hoc niche.

the blood–brain barrier^{13,37}. Diverse genes have been identified in model systems that mediate the extravasation of breast-cancer CTCs in the lungs and are also associated with lung metastasis in the clinic. These include the protein Fascin-1 and other components of invading protrusions known as invadopodia³⁹, autocrine enhancers of cancer-cell motility such as ephrins and WNT ligands^{39,40}, and mediators of endothelial disjunction and vascular permeability that include angiopoietin-like 4 (ANGPTL4), vascular endothelial growth factor (VEGF), the cyclooxygenase COX2 (also known as prostaglandin-endoperoxide synthase), MMP1 and osteonectin^{41–44}. Platelets that are associated with CTCs can stimulate extravasation by releasing TGF- β and triggering EMT in the cancer cells³² or by secreting adenine nucleotides, which relax endothelial cell junctions⁴⁵. Physical contacts between macrophages and CTCs help to pull CTCs across capillary walls in the lungs⁴⁶. Many of these factors also enhance the extravasation of CTCs in the brain, which involves the cancer-cell-derived sialyltransferase enzyme ST6GALNAC5 (ref. 47), cathepsin S⁴⁸ and microRNAs mir-105 and mir-181c^{49,50}. These mediators each provide a finite increase in the probability of metastatic seeding and frequently act in parallel. In sum, a combination of priming signals from the tumour stroma, the composition of CTC clusters, blood-circulation patterns, the structure of target-organ capillary walls and cancer-cell-autonomous functions determine metastatic infiltration of specific organs.

Tissue defences against infiltrating cancer cells

In primary tumours, cancer cells develop in a co-evolving microenvironment that suppresses immune surveillance^{17,51}. However, because support is not available immediately to cancer cells as they infiltrate distant organs, most of these cells will die^{5–7}. The ‘seed and soil’ hypothesis was based on the observation that different cancers show a predilection for metastasis in different organs and predicted that certain organs are more hospitable

to wandering cancer cells than others⁵². Seed and soil is an appealing metaphor but can be misleading. To disseminating cancer cells, every distant soil is deadly, although some soils can be less deadly than others. In fact, the most welcoming of all soils could be the primary tumour itself. The preferential reseeding of primary or metastatic tumours by CTCs, rather than the seeding of tumour-free secondary sites, is called tumour self-seeding⁵³. In experimental systems, self-seeding can amplify the most aggressive cancer-cell clones⁵³ and disperse drug-resistant clones during the treatment of metastatic melanoma with targeted therapy⁵⁴.

In a new and challenging microenvironment, freshly disseminated cancer cells can be particularly vulnerable to immune surveillance (Fig. 1). Major players in antimetastatic immune surveillance include cytotoxic T cells and natural killer (NK) cells⁵⁵. For instance, the depletion of cytotoxic T cells or NK cells has been shown to increase metastasis^{56,57} and inhibition of the tyrosine kinase Mer, a negative regulator of NK cells, suppresses metastasis⁵⁸. Moreover, the specific immune-cell composition of an organ can influence the organ’s susceptibility to overt metastasis. The liver, for example, is rich in NK cells. Neutralization of pro-apoptotic NK-cell-derived tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), or the genetic depletion of NK cells, increases hepatic metastasis in mice^{59,60}. Advances in immunotherapy, most notably those that use immune checkpoint inhibitors, have yielded striking results in metastatic melanoma and other tumour types^{61,62}. Thus, immunity is a major defence against metastasis.

Other cell types can also mount a strong defence against metastatic infiltration. Astrocytes, the most abundant type of cell in the brain, reject extravasated cancer cells by releasing the serine protease plasminogen activator (PA). PA converts the zymogen plasminogen to plasmin, which then mobilizes the pro-apoptotic cytokine Fas ligand (FasL) to kill infiltrating cancer cells. To avert this fate, brain metastatic cells from breast and lung adenocarcinomas produce the PA inhibitors neuroserpin and serpin B2 (ref. 9).

Supportive niches

Adult stem cells reside in specialized niches that provide cues that help to maintain a balance between stem-cell proliferation and quiescence as well as self-renewal and differentiation. Stem-cell niches are rich in developmental and self-renewal signals, such as hedgehog, Wnt, members of the TGF- β family and the chemokine CXCL12 (refs 63, 64). Tumours are thought to arise from mutant stem cells in their native niches or from the progeny of cells that retain their tumour-initiating capacity and benefit from these niche signals^{65–68}. After cancer stem cells disperse to distant sites, their survival and tumour-initiating potential can benefit similarly from interactions with specialized niches⁶⁹ (Fig. 2). Evidence suggests that prostate-carcinoma stem cells occupy native haematopoietic stem-cell niches in the bone marrow⁷⁰. The CXCL12 receptor CXCR4 is a marker and mediator of breast-cancer metastasis to CXCL12-rich bone-marrow sites⁷¹. Breast tumours that are rich in a CXCL12-secreting mesenchymal stroma select for CXCL12-responsive cancer-cell populations that are predisposed to survive in the bone marrow⁷².

Small blood vessels are surrounded by a space that is rich in supportive signals and can favour cancer stem-cell growth and resistance to anticancer therapy^{73,74} (Fig. 2). A striking example of the interaction of metastatic cells with perivascular sites is observed in brain metastasis by breast-cancer, lung-cancer and melanoma cells in which the extravasated cancer cells remain closely associated with capillaries^{8,75}. The cells spread along the basal lamina that surrounds the capillaries and proliferate to form a sheath that eventually engulfs and remodels the co-opted capillary network — a process that is mediated by expression of the cell-adhesion molecule L1CAM in the metastatic cells⁹ (Fig. 2).

DTCs can set up an ad hoc niche by producing components of stem-cell niches themselves (Fig. 2). Lung-metastatic breast cancer cells produce the extracellular-matrix protein tenascin C, which is deposited in the incipient colony to amplify Notch and Wnt signalling⁷⁶. Breast-cancer stem cells can also secrete TGF- β , which stimulates stromal fibroblasts to produce

periostin, a binding partner of tenascin C that recruits Wnt factors¹¹. The secretion by cancer cells of the collagen-crosslinking enzymes LOX and PLOD2, which stiffen the extracellular matrix, amplifies integrin–focal adhesion signalling and also favours metastasis^{77–79}.

Experimental models have provided evidence that systemic signals from primary tumours can influence the microenvironment of distant organs by creating pre-metastatic niches before the arrival of CTCs^{80,81} (Fig. 2). Different classes of systemic mediators, such as tumour-derived inflammatory cytokines, exosomes and extracellular-matrix-remodelling enzymes, have been shown in breast-, lung- and gastrointestinal-tumour models to recruit bone-marrow-derived cells and precondition the lung, liver or bone marrow for infiltrating cancer cells^{82–85}. Melanoma cells secrete exosomes that might induce vascular leakiness and inflammation during the formation of pre-metastatic niches⁸⁶. Similarly, macrophage inhibitory factor (MIF)-containing exosomes that are released by pancreatic cancer cells increase liver metastasis by inducing TGF- β secretion, stimulating the production of the glycoprotein fibronectin in hepatic stellate cells and recruiting bone-marrow-derived cells to the liver⁸³. Integrins were proposed to target cancer-cell-derived exosomes to specific organs to unload their cargo and prepare the organ for the arrival of tumour cells⁸⁷.

Observations from the clinic raise questions about the role of pre-metastatic niches in people with cancer. Most patients develop metastasis months or years after the removal of their primary tumour, until which time tumour cells remain largely dormant. Yet in experimental models, pre-metastatic niches support the immediate outgrowth of DTCs. Research must address whether pre-metastatic niches remain primed for years after the removal of a primary tumour or, alternatively, if their role is to enhance the survival of infiltrating cells to increase their numbers before they enter a latent state.

Growth and survival pathways

A plethora of genes and signals support metastatic cell growth and survival in experimental models, and the expression of these genes can predict relapse in the clinic (Box 1). Many such pro-metastatic stromal mediators ultimately activate stem-cell support pathways, such as the Wnt, TGF- β , bone morphogenetic protein (BMP), Notch and signal transducer and activator of transcription (Stat)3 pathways. Others activate pathways

that integrate cell metabolism and survival, including the phosphoinositide 3-kinase–protein kinase B (PI3K–AKT), mitogen-activated protein kinase (MAPK) and hypoxia-inducible factor (HIF) pathways. Positional and mechanical pathways (Hedgehog and Hippo) and inflammatory pathways (NF κ B and Stat1) are also activated⁶⁹. These pathways also drive development and tissue regeneration, but what is distinctive in the case of metastasis are the strategies that cancer cells employ to ensure that sufficient pathway activation is achieved in microenvironments with low levels of activating signals (Fig. 3). DTCs seem to be selected according to their ability to use whatever cues the host tissue offers.

By expressing autocrine pathway activators or by recruiting stromal cells to produce them (Fig. 3), DTCs can stimulate a crucial pathway or amplify the pathway's signalling output⁶⁹. For example, Stat3 stimulation by autocrine interleukin (IL)-6 mediates metastasis in prostate cancer cells, and PI3K–AKT stimulation by autocrine insulin-like growth factor 2 (IGF2) mediates metastasis in oesophageal cancer cells^{88,89}. The intracellular tyrosine kinase Src amplifies the ability of stromal CXCL12 to activate PI3K–AKT signalling in breast cancer cells that infiltrate the bone marrow⁹⁰. Breast cancer cells produce colony-stimulating factor 1 (CSF1) to recruit tumour-associated macrophages as a source of epidermal growth factor (EGF)⁹¹ or secrete CXCL1 to recruit myeloid precursors as a source of calprotectin (S100A8/9) for MAPK activation⁹². Colorectal-cancer stem cells that reach the liver express TGF- β to recruit mesenchymal cells as a source of IL-11 for Stat3 activation in the cancer cells¹².

Cancer cells can also obtain vital support through physical contact with stromal cells (Fig. 3). Claudin-2-mediated cell–cell interactions between breast cancer cells and hepatocytes induce c-Met (hepatocyte growth factor receptor) signalling and stimulate metastasis to the liver⁹³. Contact between membrane vascular cell adhesion molecule 1 (VCAM1) on breast cancer cells that have infiltrated the lungs and α 4 integrins on stromal monocytes and macrophages activates PI3K–AKT signalling in the cancer cells⁹⁴. By contrast, contact between VCAM1 from breast cancer cells that exit dormancy in the bone marrow and α 4 integrins on monocytic precursors accelerates the differentiation of these precursors into osteoclasts that mediate osteolytic metastasis⁹⁵.

The activity of pro-metastatic pathways can be further increased by epigenetic alterations. For example, von Hippel–Lindau (VHL)-mutant renal-cell-carcinoma cells gain metastatic activity in multiple organs

BOX 1

Origin of metastatic traits

Metastasis develops through genetic and epigenetic changes and subsequent selection for favourable traits under the pressure of successive bottlenecks^{138,139}. Genomic comparisons show close clonal relationships between primary tumours and their metastases. Specific ancestors of metastatic clones can often be identified in the primary tumour^{30,139,140}, which supports the hypothesis that late clonal expansion in the primary tumour gives rise to metastasis-competent clones. These studies also provide evidence that metastases seed further metastases³⁰. Disseminated cancer cells remain dependent on the oncogenic mutations that underlie the primary tumour, which provides a basis for treating metastasis with drugs that target these oncogenic drivers¹²⁵. Oncogenic mutant alleles accumulate in metastases, such as the gain of mutant *KRAS* in pancreatic cancer metastasis¹⁴¹ and *TP53* and androgen-receptor mutations in prostate cancer metastasis¹⁴². However, no recurrent metastasis-specific mutations have been identified so far, which suggests that epigenetic alterations and other mechanisms of modifying gene expression are the predominant source of selectable pro-metastatic traits during clonal evolution in metastasis^{139,140}.

The cell-autonomous traits that favour the dissemination of cancer cells, their resistance in the circulation, extravasation and initial survival

in distant organs become important as soon as the cells leave the primary tumour, and are pre-selected in the primary tumour. For example, certain mediators of neoangiogenesis in breast tumours, such as COX2, epiregulin, MMP1 and VEGF, are repurposed by cancer cells for extravasation in the lungs and brain^{42,47,143}. Stromal TGF- β in triple-negative breast carcinomas — which do not express the oestrogen receptor, progesterone receptor or HER2 — induces the expression of the protein angiopoietin-like 4 (ANGPTL4) and primes the cancer cells for extravasation in the lungs⁴¹. These early metastatic traits can be selected under the stresses of tissue invasion, immune surveillance or hypoxia. The evidence favours a model in which a considerable proportion of cancer cells in a primary tumour acquire pro-metastatic traits that confer a finite probability of success in the early steps of metastasis. Clones with the most effective combination of pro-metastatic traits are most likely to give rise to metastatic lesions and also to re-seed the primary tumour. Beyond these early steps, cancer cells continue to evolve after their dissemination to distant organs to acquire traits for overt colonization, as demonstrated in bone metastasis¹¹⁴. Cancer cells that undergo early dissemination could evolve in parallel with, but independently from, the primary tumour¹⁴⁴. The origin of metastatic traits remains a fertile area for future research.

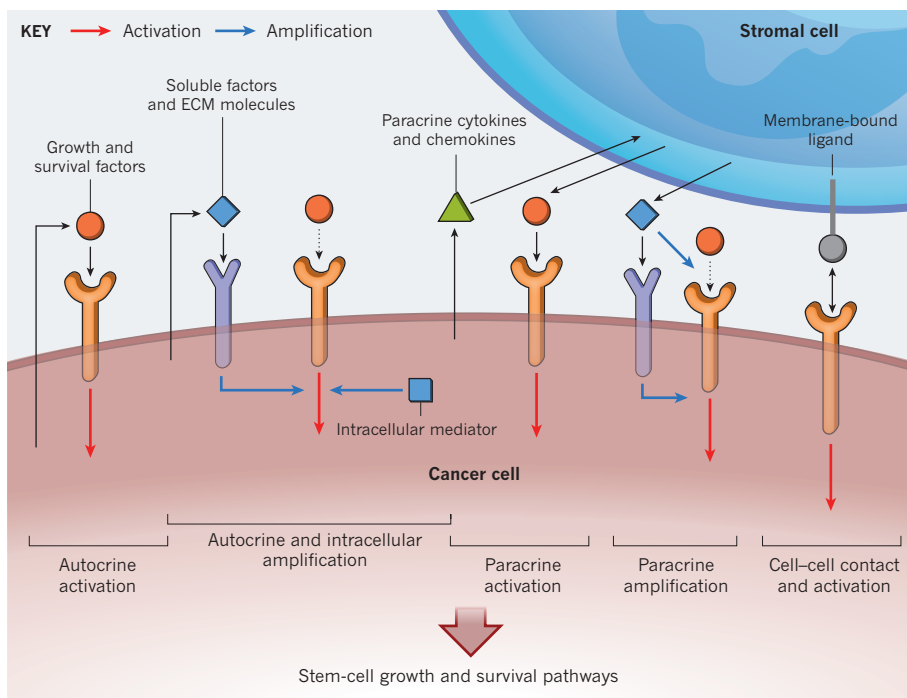


Figure 3 | The activation of growth and survival pathways by disseminated cancer cells. During colonization, metastasis-initiating cells require the activity of a common set of pathways that support the survival and growth of stem and progenitor cells. After infiltrating distant tissues, the disseminated cancer cells express paracrine mediators (such as IL-6 and IGF2), ECM components (such as TNC, LOX and PLOD2) and intracellular mediators (such as SRC and Ezrin) that activate and amplify survival and growth pathways. They also express paracrine factors (such as CSF1, CXCL1 and TGF- β) that help to recruit stromal cells as a further source of soluble activators and amplifiers that include the cytokines IL-6, WNT, EGF and S100A8/9 and the ECM components TNC and POSTN. Cancer cells can also achieve pathway activation through cell–cell contact using receptor–ligand pairings such as VCAM1- α 4 integrin and Jagged1–Notch.

through changes in DNA methylation and histone acetylation that expand the range of HIF target genes — the dominant oncogenic pathway in these cells⁹⁶. Additional inputs come from the expression of microRNAs that promote or suppress metastasis by regulating multiple mediators of tumour–stroma interactions^{97–100}. These examples show that DTCs resort to diverse mechanisms to procure vital inputs for survival and the retention of their tumour-initiating capacity.

Exactly when, where and how cancer cells turn to these various stromal cues is unclear. Are these niches and pathways important for all stages of metastatic colonization, at all times? Some might be crucial only after extravasation, when cancer cells are challenged by tissue defences or during the latent phase of metastasis in which cancer cells subsist for years without outgrowth. Yet others might be important only when DTCs exit dormancy and enter a proliferative state. Such questions remain unanswered because most experimental models of metastasis do not incorporate a latency phase. This gap in knowledge is also of concern from a translational perspective. Treating overt metastasis by targeting a survival mechanism that was relevant only during the initial infiltration of distant organs might have no clinical benefit. Likewise, targeting an oncogenic driver pathway in latent DTCs could be futile. But targeting mechanisms that support the viability of latent DTCs could actually eradicate residual disease.

Latent metastasis

The observation that patients relapse with metastatic disease months or years after removal of their primary tumour, combined with the detection of DTCs in the bone marrow of people with no evidence of metastatic disease, demonstrates that cancer cells that disseminate before treatment of the primary tumour retain the ability to initiate metastatic growth long thereafter. Some organs are more permissive than others to the accumulation of latent DTCs. For example, people with colorectal or gastric cancer can harbour DTCs in the bone marrow, yet the incidence of bone metastasis in these patients is low¹⁰¹. The incidence of DTCs in the bone marrow is a predictor of metastasis not only to bone, but also to the liver, lungs and brain².

Tumour dormancy is thought to occur through two modes (Fig. 1). In cellular dormancy, isolated DTCs enter a state of proliferative quiescence. Indeed, most DTCs in bone-marrow samples from people with cancer are found as quiescent single cells^{101–103}. But in tumour mass dormancy, micrometastases cease to grow because of insufficient vascularization or

constant culling by immune defences¹⁰². It is uncertain which mode most frequently leads to overt metastases.

Despite the biological and clinical relevance of metastatic latency, little is known about the mechanisms by which cancer cells enter a dormant state and the signals that sustain it, the niches that dormant cancer cells inhabit and what triggers the resumption of the cells' aggressive growth. The paucity of experimental model systems that incorporate a latent phase and the cost of studying such a process over an extended period in animal models have hindered progress. However, stromal signals that impose tumour dormancy have been identified in mouse xenograft models. TGF- β and BMPs, members of the TGF- β family, can enforce quiescence and inhibit self-renewal in carcinoma DTCs^{104–106}. The perivascular niche has also been implicated in the induction of cancer-cell dormancy¹⁰⁷. But environments that are rich in type I collagen¹⁰⁸ or fibronectin¹⁰⁹ inhibit dormancy.

A scarcity of stromal growth factors and an abundance of growth-inhibitory cues can favour metastatic dormancy in experimental models. On their own, however, these signals might not be able to sustain long-term metastatic latency. Tissues that host DTCs, such as the lungs, liver or bone marrow, do not exist in a perpetual state of growth inhibition. On the contrary, they support cell proliferation as part of normal tissue homeostasis and regeneration in a context that would regularly stimulate DTCs to enter the cell cycle. Therefore, cancer-cell-autonomous mechanisms that self-impose quiescence might be necessary to maintain DTCs in a dormant state. It is also unclear how a continuously quiescent DTC population could evolve and acquire the necessary traits for overt metastasis.

Evidence that DTCs are kept in a latent state by the immune system comes from the transmission of cancer during organ transplantation. Kidney, liver and lung transplants from donors who were cured of melanoma or who had developed glioblastoma, which is generally considered to be a nonmetastatic tumour, have resulted in the transmission of donor-derived tumours to immunosuppressed recipients^{110,111}. Such cases suggest that DTCs are maintained in a latent state by constant pressure from the immune system. DTCs might enter the cell cycle intermittently, and their progeny might undergo rapid elimination by the immune system while evolving to acquire traits that facilitate metastatic outbreak.

Overt metastasis

In some organs, breaking down growth-inhibitory or immune barriers can be sufficient to initiate the aggressive metastatic outgrowth of DTCs.

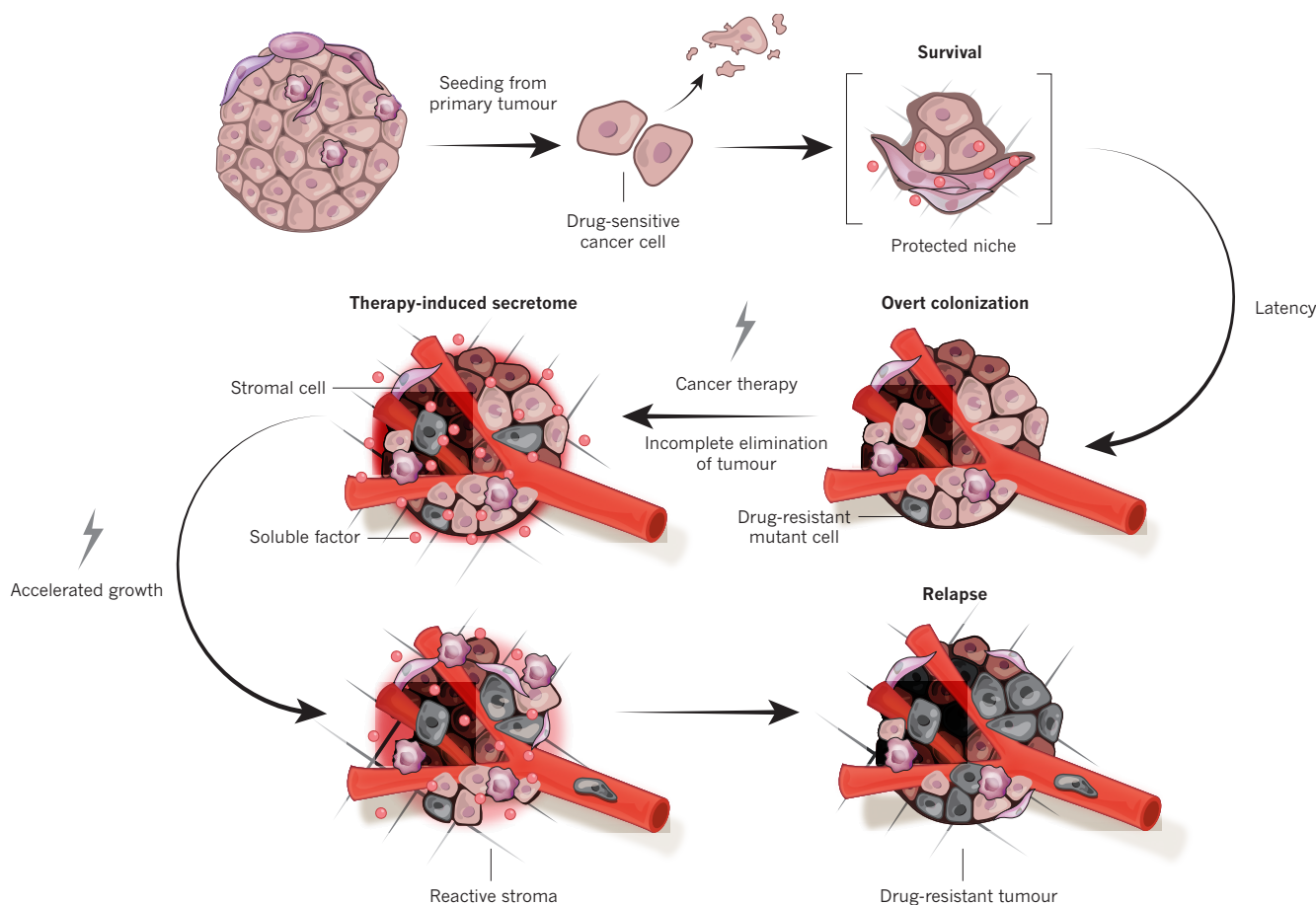


Figure 4 | The biology of metastasis, before and after cancer therapy.

Latent metastasis occurs owing to conditions that preserve the survival and tumour-initiating ability of disseminated cancer cells. The elimination of latent metastasis by targeting these survival mechanisms would prevent metastasis. Cancer cells that exit latency form manifest metastases. This condition is treated with combinations of conventional chemotherapy, targeted therapy and immunotherapy. Although treatment can dramatically reduce the metastatic burden, tumour elimination is frequently incomplete. A considerable

proportion of the tumour cell population will withstand treatment by adapting its intracellular pathways or by activating supportive paracrine inputs. The stress of targeted therapy causes drug-sensitive cancer cells to express a large number of secreted factors, known as a therapy-induced secretome, that can salvage drug-sensitive cells and accelerate the growth of minority drug-resistant cancer cells. This accelerated growth drives relapse as a drug-resistant tumour. The survival and growth mechanisms that residual cancer cells use during cancer therapy might resemble those used by their predecessors in the latent phase.

However, organs differ markedly in the structure and composition of their tissues, and their overt colonization involves distinct organ-specific metastatic traits¹¹². This translates into patterns of metastasis distribution that vary greatly depending on the tumour type. For example, prostate cancer has a propensity to relapse in bone; uveal melanoma tends to recur in the liver; and sarcomas often return in the lungs. In contrast, melanomas, breast carcinomas and lung adenocarcinomas tend to relapse in multiple organs. The kinetics of relapse also varies. For example, whereas recurrence in the brain frequently occurs at an early stage in metastatic lung cancer, this is typically a late event in metastatic breast cancer. Certain oncogenic mutations seem to affect metastatic tropism. For example, *KRAS*-mutant colon cancer advances to colonize the lungs from established liver metastases¹¹³.

Bone metastasis is the best-understood case of overt colonization and offers clear examples of the organ-specific traits that determine this final stage of metastasis. Osteolytic bone metastasis occurs when the balance of bone-generating and bone-resorbing osteoclasts is altered to favour the latter. Numerous mediators of osteoclast activation have been implicated in this process¹¹⁴. Cancer-cell-derived parathyroid hormone-related protein (PTHrP), IL-11 and TNF- α prompt osteoblasts to release receptor activator of NF κ B ligand (RANKL), a protein that stimulates osteoclast maturation^{114–116}. Bone metastatic cells also produce MMPs, which increase RANKL activity¹¹⁷ and reduce the levels of the RANKL antagonist osteoprotegerin¹¹⁸. Expression of the Notch ligand

Jagged1 and cell-adhesion molecules VCAM1 and soluble intercellular adhesion molecule 1 (sICAM1) also contributes to the mobilization of osteoclasts^{95,119,120}. Bone-matrix degradation by hyperactivated osteoclasts releases TGF- β that, in turn, augments the production of PTHrP, IL-11 and Jagged1 in the cancer cells and drives a vicious cycle of bone destruction^{114–116}.

Interestingly, prostate cancer cells that spread to bone alter the homeostatic balance in favour of osteoblastic activity, which stimulates the deposition of bone matrix and leads to eventual displacement of the bone marrow. Cancer-cell factors that are implicated in osteoblastic metastasis include fibroblast growth factors (FGFs), insulin-like growth factors (IGFs) and VEGFs, as well as endothelin 1, Wnt factors and BMPs¹¹⁴. Thus, bone metastasis provides a compelling example of how cancer cells engage their host microenvironment in overt metastasis. Similarly, specific stromal components can be engaged in other organs by metastatic cells with the necessary organ-specific colonization traits. For example, when breast and lung carcinomas spread to the brain, the cancer cells can profitably engage astrocytes and microglia by expressing endothelin 1 (ref. 121). However, our knowledge of overt-colonization traits that are specific to organs other than bone is limited, and these traits need further investigation.

In certain groups of patients, metastasis is confined mainly to a particular organ that is better able to resist therapy than others. An example is the rise in the incidence of late brain and leptomeningeal

metastasis in people with HER2⁺ breast cancer. Such patients can benefit from advances in targeted therapies that suppress extracranial metastasis. However, this success is often short-lived owing to the emergence of brain metastasis. Brain metastasis is a major cause of morbidity and mortality, with an overall incidence that is ten times higher than that of all primary brain tumours combined, and has few therapeutic options. A better understanding of its underlying mechanisms is urgently needed.

After therapy

The surgical removal of a malignant tumour is often complemented with radiotherapy and systemic chemotherapy to suppress relapse. If metastasis becomes clinically manifest, most systemic treatments are designed to target metastasis irrespective of organ site. Treatments include classic chemotherapy, targeted therapy against oncogenic drivers, immunotherapeutic agents that leverage the antitumour power of the immune system and, increasingly, a combination of all of these. Treatments that target metastasis in a particular organ — by taking aim at cancer-cell interactions with the host tissue — would be indicated when metastasis is confined to that organ, as is the case with bone metastasis in some people with breast cancer. A meta-analysis suggests that adjuvant therapy with osteoclast-inhibitory bisphosphonates suppresses bone metastasis and prolongs survival in postmenopausal women with breast cancer¹²². Denosumab, an antibody that targets RANKL, reduces the incidence of bone fractures associated with metastasis in patients who are receiving aromatase inhibitors¹²³.

Despite these advances, therapies frequently achieve only partial tumour shrinkage and leave behind substantial amounts of disease. Continued treatment can keep the residual tumour indolent for some time. However, drug-resistant cancer-cell clones eventually emerge that drive rapid relapse^{124,125}. As a result, the cure rates of patients with metastasis remain disappointingly low.

Research is beginning to focus on the biology of residual metastatic cells after therapy, and is aiming to better suppress the re-emergence of cancer cells (Fig. 4). The cancer-cell population can resist treatment through alterations to negative-feedback signalling loops¹²⁶ and supportive interactions with the tumour microenvironment. For example, DNA-damaging agents induce the secretion of trophic factors such as IL-6 and the metalloproteinase inhibitor Timp-1 in normal cells of the thymus, which creates a chemoprotective niche for the survival of residual cancer cells that facilitates eventual relapse¹²⁷. Similarly, stromal fibroblasts secrete Wnt16b in response to chemotherapy, which promotes resistance to therapy in prostate cancer¹²⁸. Chemotherapy-induced expression of TNF- α in tumour-associated endothelial and mesenchymal cells has been shown to amplify the expression of the pro-metastatic cytokine CXCL1 in cancer cells⁹². In BRAF-mutant melanomas that are treated with RAF inhibitors, tumour-associated macrophages secrete TNF- α and VEGF^{129,130}. Tumour-associated fibroblasts secrete hepatocyte growth factors (HGFs)¹³¹, which protect melanoma cells and limit the effectiveness of therapy.

Under the stress of therapy, the cancer cells themselves can be a source of survival signals^{54,132,133}. Targeted therapy with tyrosine-kinase inhibitors that is directed against melanoma (vemurafenib and dabrafenib) or lung adenocarcinoma (erlotinib and crizotinib) triggers the production of a complex secretome — the therapy-induced secretome — that activates multiple survival pathways in the remaining, drug-sensitive cancer cells⁵⁴. Furthermore, this secretome can stimulate the outgrowth, dissemination and further metastatic seeding of clones with mutations that confer drug resistance. Collectively, these findings reveal a complex biology in cancer cell populations that remain after treatment of metastatic tumours and contribute to tumour relapse.

Future directions

An important objective for current research is the identification of mediators of metastasis that are common to different organs and types of tumours. Although organ-specific metastasis has intrigued researchers for over a century, in reality many patients are affected by, or are at

risk of, metastasis in multiple organs. For these cases, it would be valuable to identify common mediators as potential therapeutic targets. For example, checkpoint immunotherapy — which has shown encouraging success in the clinic — is based on the premise that immune evasion is a shared feature of metastatic disease, irrespective of the organ site. Deeper understanding of the common mediators of metastasis and how tumours regrow after therapy would help to improve strategies for the elimination of residual disease.

The advent of single-cell analysis techniques, such as single-cell RNA sequencing and signalling-pathway profiling, is allowing functional and phenotypic analysis of heterogeneous cell populations in unprecedented detail^{134–137}. The application of these techniques to residual disease and overt metastases will enable researchers to define tumour heterogeneity, cell-population structures and evolution, and cell-type-specific response patterns to stromal cues and therapeutic agents, as well as other parameters, at a depth never before possible. Furthermore, the ability to analyse circulating tumour DNA in the blood of people with cancer will facilitate the monitoring of therapy responses, the emergence of distinct resistant clones and patterns of early disease recurrence.

Preventing metastasis in high-risk patients would be far better than having to treat it. The systemic nature of metastatic disease, the heterogeneity of metastatic tumours, the multitude of genes and pathways involved in different organs and the many mechanisms of drug resistance paint a sobering picture of the problems that must be overcome to address overt metastatic disease. Ostensibly, the goal of systemic therapy that is delivered after removal of a primary tumour is to prevent relapse. However, most agents used in adjuvant therapy are designed to target growing cancer cells rather than the quiescent DTCs that predominate during metastatic latency. A better understanding of the basis for metastatic colonization and its latent phase, in particular, is needed to develop superior treatments. Research on the mechanisms that support the viability of latent metastatic cells should yield clues for targeting residual disease, with the goal of preventing metastasis. ■

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