

DISSEMINATION AND GROWTH OF CANCER CELLS IN METASTATIC SITES

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Metastases, rather than primary tumours, are responsible for most cancer deaths. To prevent these deaths, improved ways to treat metastatic disease are needed. Blood flow and other mechanical factors influence the delivery of cancer cells to specific organs, whereas molecular interactions between the cancer cells and the new organ influence the probability that the cells will grow there. Inhibition of the growth of metastases in secondary sites offers a promising approach for cancer therapy.

METASTASIS

Metastases arise following the spread of cancer from a primary site and the formation of new tumours in distant organs. When cancer is detected at an early stage, before it has spread, it can often be treated successfully by surgery or local irradiation, and the patient will be cured. However, when cancer is detected after it is known to have metastasized, treatments are much less successful. Furthermore, for many patients in whom there is no evidence of metastasis at the time of their initial diagnosis, metastases will be detected at a later time. These metastases can show an organ-specific pattern of spread — for example, **breast** and **prostate cancer** often metastasize to **bone** — that might occur years or even decades after apparently successful primary treatment.

Surprisingly though, in spite of the clinical importance of metastasis, much remains to be learned about the biology of the metastatic process. In part, knowledge is limited because metastasis is a 'hidden' process, which occurs inside the body and so is inherently difficult to observe. Many molecular factors have been identified as contributing to the formation of detectable metastases, and additional factors will rapidly be identified from studies that use microarray expression profiling. However, the identification of molecules and genes that are associated with a metastatic end point does not, in itself, provide information about how these molecules contribute to the metastatic process. Studies using *in vivo* video microscopy, and quantitative approaches that follow the fate of cancer cells in the body, are shedding light on this 'occult' process. This knowledge

will be important for providing the biological context in which to apply the rapidly increasing information about molecular contributors to metastasis. So, which metastatic steps are inefficient, and how can we use this knowledge to better design cancer therapies?

Steps in the metastatic process

The metastatic process consists of a series of steps (FIG. 1), all of which must be successfully completed to give rise to a metastatic tumour^{1–4}. As a primary tumour grows, it needs to develop a blood supply that can support its metabolic needs — a process called ANGIOGENESIS. These new blood vessels can also provide an escape route by which cells can leave the tumour and enter into the body's circulatory blood system⁵ — known as intravasation. Tumour cells might also enter the blood circulatory system indirectly via the lymphatic system. The cells need to survive in the circulation until they can arrest in a new organ; here, they might extravasate from the circulation into the surrounding tissue. Once in the new site, cells must initiate and maintain growth to form pre-angiogenic micrometastases; this growth must be sustained by the development of new blood vessels in order for a macroscopic tumour to form.

The process of metastasis has been modelled experimentally. In these assays, cancer cells are injected into experimental animals, either ORTHOTOPICALLY or directly into the circulation to model the latter phases of the process. These assays are called, respectively, spontaneous or experimental metastasis

ANGIOGENESIS

The formation of new blood vessels that are needed for growth of primary tumours and metastases that are beyond a minimal size.

ORTHOTOPIC INJECTION

Injection of cancer cells into the 'correct' anatomical site for primary tumour growth — for example, mammary fat pad for breast cancer cells.

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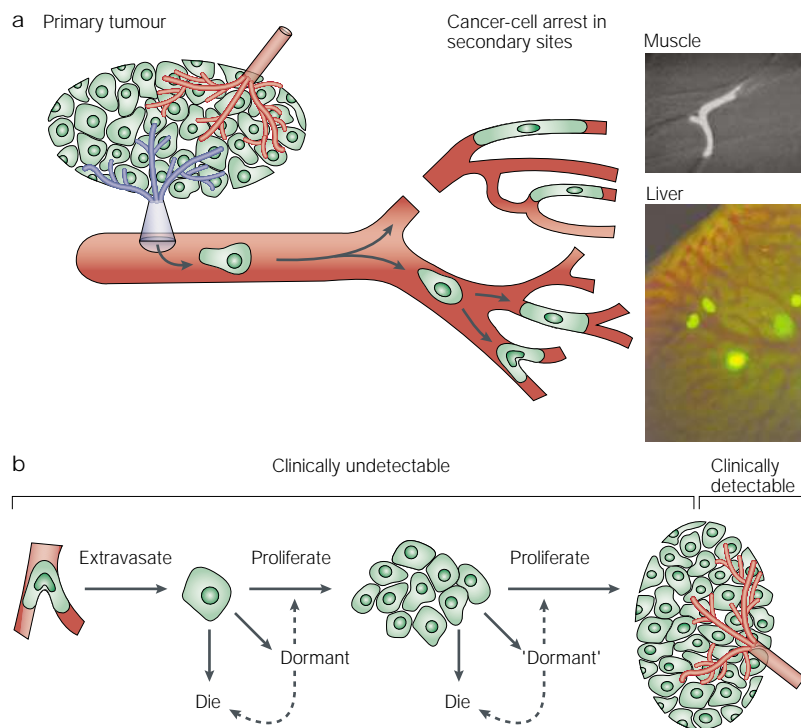


Figure 1 | The metastatic process. a | Escape of cancer cells from a primary tumour, and arrest in secondary sites. Cells that are able to escape from a primary tumour into the blood circulation are then carried by the flow to secondary sites, where they are arrested by size restriction in small capillaries in the new organ. Cancer cells are deformed to fit the vasculature in the new sites, depending on the blood pressure in the new organ. Examples shown are cells arrested in muscle, where blood pressure is high, and in liver, where the pressure is lower. (Cells also might escape from a primary tumour into lymphatic channels in the tumour — see FIG. 2). **b** | Possible fates of cancer cells in a secondary site, following the arrival of circulating cancer cells in an organ. Cancer cells can exist in a secondary site as solitary cells, small pre-angiogenic metastases or larger vascularized metastases. At each step, only a subset will proceed, and the remainder of cells or micrometastases might either go into a state of dormancy, or die. Only a proportion of vascularized metastases are clinically detectable, and solitary cells and micrometastases are generally clinically undetectable. Dormant solitary cells refer to cells that are undergoing neither proliferation nor apoptosis, whereas ‘dormant’ pre-angiogenic micrometastases refer to those in which active proliferation is balanced by active apoptosis, resulting in no net increase in the size of the metastases.

assays^{6,7}, and the end point of both assays is the formation of visible metastases at a secondary site. These assays have led to the identification of many molecular alterations in cancer cells that can contribute to their ability to metastasize (see REFS 8–14 for reviews). However, end-point assays are poorly able to clarify which steps in the metastatic process are affected by specific molecules^{11,15}. For example, it was assumed early on that matrix metalloproteinases (MMPs), which had been shown by many end-point metastasis assays to affect metastatic outcome, must have a primary effect on facilitating the escape of cancer cells from the circulation. However, *in vivo* videomicroscopy studies — either using cells transfected to overexpress the MMP inhibitor TIMP1 (tissue inhibitor of metalloproteinase 1), or treating mice with the MMP inhibitor BB-94 — showed that MMPs have a much broader role in the metastatic process and have a significant effect on the ability of cancer cells to grow in a secondary site^{11,16,17}.

Quantitative studies using *in vivo* videomicroscopy and cell-fate analysis to determine the efficiency of each step in the metastatic process have revealed several surprises about the biology of the process of metastasis¹⁸, which provide a physical and biological context in which to interpret molecular findings.

Organ-specific metastasis: ‘seed and soil’
It has long been recognized that some types of cancer show an organ-specific pattern of metastasis. Breast cancer frequently metastasizes to bone, liver, brain and lungs; prostate cancer preferentially spreads to bone. Patients with colorectal cancer, by contrast, often develop initial metastases in liver.

In 1889, Stephen Paget published an article in *The Lancet* that described the propensity of various types of cancer to form metastases in specific organs, and proposed that these patterns were due to the ‘dependence of the seed (the cancer cell) on the soil (the secondary organ)’^{19–21}. This idea was challenged in the 1920s by James Ewing, who suggested that circulatory patterns between a primary tumour and specific secondary organs were sufficient to account for organ-specific metastasis²² (BOX 1).

In fact, these theories are not mutually exclusive, and current evidence supports a role for both factors. In a series of autopsy studies^{23–26}, Leonard Weiss documented that larger numbers of bone metastases than would be expected based solely on blood-flow patterns were identified for both breast and prostate cancer, for example. By contrast, fewer numbers of skin metastases than expected based on blood-flow patterns were found for osteosarcomas, stomach and testicular cancers. Of the 16 primary tumour types and 8 target organs that were analysed, metastasis in 66% of the tumour-type–organ pairs seemed to be adequately explained on the basis of blood flow alone, whereas the remainder were not. For these remaining tumours, negative interactions — that is, fewer metastases than expected based on blood flow between the primary and secondary sites — between cancer cells and the environment of the metastatic site were found in 14% of cases, and positive interactions were found in 20% of cases²⁶.

Experimental data from metastasis assays in laboratory mice also support the concept that both mechanical factors (how many cells are delivered to an organ?) and seed–soil compatibility factors (does the organ preferentially support or suppress the growth of the specific cancer-cell type?) contribute to the ability of specific types of cancer to spread to various target organs^{27–29}.

Vascular pathways affect metastatic spread. FIGURE 2 shows the blood-flow pathways for cancers growing in two different primary sites: breast and colon. Breast cancer cells that escape from the primary site into the blood circulation would be taken by the flow through the heart to the capillaries of the lungs, where many would arrest. Any cells that managed to pass through these capillaries would then enter the systemic arterial circulation, where they would be taken to capillary beds in all organs of the body.

Summary

- Metastasis consists of a series of sequential steps, all of which must be successfully completed. These include shedding of cells from a primary tumour into the circulation, survival of the cells in the circulation, arrest in a new organ, extravasation into the surrounding tissue, initiation and maintenance of growth, and vascularization of the metastatic tumour.
- Some types of tumour show an organ-specific pattern of metastasis. Both 'seed' (the cancer cell) and 'soil' (factors in the organ environment) contribute to this organ specificity.
- Mechanical factors influence the initial fate of cancer cells after they have left a primary tumour. Blood-flow patterns from the primary tumour determine which organ the cells travel to first. There, the relative sizes of cancer cells and capillaries lead to the efficient arrest of most circulating cancer cells in the first capillary bed that they encounter.
- After cells have arrested in an organ, their ability to grow is dictated by molecular interactions of the cells with the environment in the organ.
- Metastasis is an inefficient process. *In vivo* videomicroscopy and cell-fate analysis have led to the conclusion that early steps in metastasis are completed very efficiently. By contrast, later steps in the process are inefficient. Metastatic inefficiency is due primarily to the regulation of cancer-cell growth in secondary sites.
- Metastases can occur many years after primary cancer treatment. Tumour dormancy might be due to pre-angiogenic micrometastases that subsequently acquire the ability to become vascularized, or solitary cells that persist for an extended period of time without division in a secondary site. These cells would be resistant to current cancer therapies that target actively dividing cells.
- Because growth of metastases is a primary determinant of metastatic outcome, the growth phase of the metastatic process is a promising therapeutic target. Treatments that target the specific 'seed-soil' compatibility that results in organ-specific metastatic growth would be especially useful.

Box 1 | 'Seed' or 'Soil'

Stephen Paget (1855–1926) was an English surgeon — son of the famed surgeon Sir James Paget. Stephen Paget trained at St Bartholomew's Medical School, and then practiced surgery in London. He developed a strong interest in supporting research into cancer, and in 1908 he founded the Royal Defense Society to provide scientific input into the animal-welfare debate and to support the need for animal research for the benefit of cancer patients. On the basis of his numerous observations of cancer patients, he published an article in 1889 in *The Lancet*, which commented on the propensity of some types of cancer to give rise to secondary growth ('metastasis') in specific organs. This paper led to the formulation of the 'seed and soil' theory of cancer spread.

The following are some key quotes from this 1889 paper¹⁹:

"An attempt is made in this paper to consider 'metastasis' in malignant disease, and to show that the distribution of the secondary growths is not a matter of chance."

"What is it that decides which organs shall suffer in a case of disseminated cancer?"

"When a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil."

James Ewing (1866–1943) was an American pathologist who trained at the College of Physicians and Surgeons in New York. He was appointed as the first Professor of Pathology at Cornell University in New York in 1899 — a post he held for the following 33 years. He was a leading cancer pathologist and also maintained an active interest in cancer research. He co-founded the American Association for Cancer Research and the current American Cancer Society, and was also instrumental in establishing the Memorial Sloan-Kettering Cancer Center. He wrote the successful textbook *Neoplastic Diseases*. In the text, he actually says relatively little about the role of 'seed' and 'soil' in metastasis; the following is a quote from the metastasis chapter that deals with this subject²²:

"'Genius loci,' or the particular susceptibility of a tissue to develop secondary tumors, is an interesting phase of study of metastases...The mechanisms of the circulation will doubtless explain most of these peculiarities, for there is as yet no evidence that any one parenchymatous organ is more adapted than others to the growth of embolic tumor cells. The spleen seems to escape with peculiar frequency."

Alternatively, some breast cancer cells might invade lymphatics in the primary site and would be taken first to the draining lymph node, where they might grow (FIG. 2). From the lymph nodes, however, there are no direct lymphatic routes to the sites where breast cancer metastases are often found — bone, liver, brain and lungs — so cells in the lymphatic system would eventually need to enter the blood circulation to be transported to these sites. This could occur indirectly, through efferent lymphatic vessels that eventually flow into the venous system, or directly into newly formed blood vessels that serve lymph-node metastases. Although lymph-node metastasis is a negative prognostic factor for breast and other cancers, it is still not known whether metastasis to other organs proceeds sequentially from lymphatic spread or in parallel by a HAEMATOGENOUS route.

In contrast to the spread patterns for breast cancer cells by means of the venous system, cancer cells leaving a primary colon tumour would enter the hepatic-portal system and would be taken first to the capillaries (sinusoids) of the liver (FIG. 2). Any cells that were able to pass through this first capillary bed would then be taken via the venous circulation through the heart to the capillaries of the lung, and access to the arterial circulation (and other organs of the body) could occur if any cells were then able to pass through the capillaries of the lung. So, cells leaving a tumour will be taken preferentially to specific organs, depending on blood-flow patterns, and the initial steps in the metastatic process depend on these patterns¹⁸.

*Do cancer cells 'home' to specific organs? How efficient are capillaries at 'filtering' out these circulating cancer cells? Studies using *in vivo* video microscopy and*

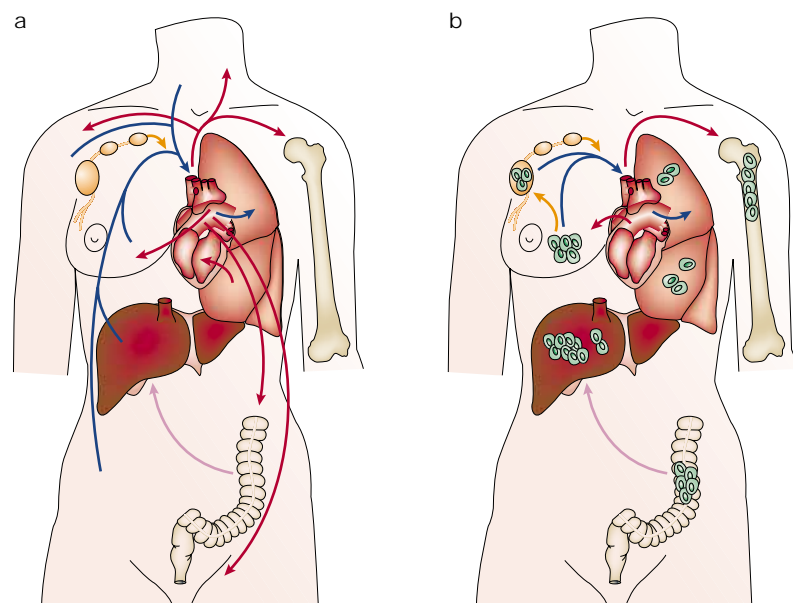


Figure 2 | Vascular flow patterns and corresponding movement of cancer cells arising in different organs. a | Blood from most organs of the body is carried directly to the heart by the venous system and passes to the lungs (blue). It then returns to the heart and is circulated to all organs of the body by the systemic arterial system (red). Blood from splanchnic organs, such as the intestines, passes first to the liver (pink) before entering the venous system. Throughout the body, excess extravascular fluid enters lymphatic vessels (yellow), passes through lymph nodes and is returned to the venous system. **b** | Breast cancer cells that leave the primary tumour by blood vessels will be carried by the blood flow first through the heart and then to the capillary beds of the lungs. Some cancer cells might pass through the lung to enter the systemic arterial system, where they are transported to remote organs, such as bone. Others might form metastases in the lung, which might then shed cells to the arterial flow. By contrast, colon cancer cells will be taken by the hepatic-portal circulatory system first to the liver. There is no direct flow from the lymphatic system to other organs, so cancer cells within it — for example, breast cancer cells — must enter the venous system to be transported to remote organs.

quantitative cell-fate analyses indicate that both lung and liver are very efficient at arresting the flow of cancer cells^{4,15,18,30–32}, and that most circulating cancer cells arrest by size restriction (FIG. 1a,3a). Capillaries are small (typically 3–8 μm in diameter³³) and are designed to allow the passage of red blood cells — which average 7 μm in diameter and are highly deformable — whereas many cancer cells are quite large (20 μm or more in diameter). The actual percentage of circulating cancer cells that arrest by size restriction in any given organ will be determined by physical factors, such as the relative sizes of the cells and the capillaries, the blood pressure in the organ and the deformability of the cell. By contrast, white blood cells (WBCs), which are smaller than many cancer cells, are carried by the blood flow through the capillaries into the venules, where WBCs often arrest by adhering to the walls of vessels that are much larger in diameter than the cells themselves (FIG. 3b). This type of adhesive interaction — mediated by selectins and integrins — has been well described³⁴, but cancer cells do not usually arrest in this manner when injected into normal mice^{4,14,18,30–32,35,36}. However, cancer cells can undergo adhesive arrest in the liver in pre-capillary vessels (portal venules) that are larger than the cell diameter,

when the endothelium has been activated by the cytokine interleukin (IL)-1 α ³⁷. This treatment would not cause a change in the total proportion of cells that arrest in the organ, but would merely cause more cells to be arrested in the organ before reaching the small capillaries. So, given the very high initial arrest of cancer cells in the first capillary bed they encounter, it is reasonable to propose that organ-specific adhesive interactions are indicative of organ-specific signalling, rather than factors that enhance the physical arrest of cancer cells in specific sites.

Unlike WBCs, which can recirculate until they arrive at a site that is conducive to adhesion, most cancer cells that leave a primary tumour do not have the opportunity to seek out a 'compatible' secondary site. Instead, they are taken passively to the first (or perhaps the second) capillary bed they encounter — based on blood-flow patterns from the primary tumour site — and then most of them are arrested there by size restriction. Although cancer cells are sometimes said to 'home' to specific organs — based on the end point of detectable metastases in those organs (for example, breast tumours metastasizing to bone) — it is more likely that this organ specificity is due to efficient organ-specific growth rather than preferential 'homing' of cells to a particular organ. So, the initial delivery and arrest of cancer cells to specific organs seems to be primarily 'mechanical'. Once cells have been 'seeded' to an organ, however, their subsequent growth will depend on the compatibility of the 'seed' with the 'soil' that they encounter in the organ. This growth regulation must depend on the molecular interactions between cancer cells and the environment of the new organ.

Molecular regulation of metastatic growth. A considerable amount of evidence indicates that molecular factors that are present in specific organs can, indeed, influence whether or not various types of cancer cell will grow there^{29,38–41}. These studies indicate that tumour cells will respond quite differently — in terms of gene expression, growth ability, responsiveness to therapy and so on — depending on the environment that they encounter in the new organ. So, any breast or prostate cancer cells that happen to arrive in bone might be stimulated to grow with high efficiency because of reciprocal molecular interactions that occur between the cancer cells and cells in the bone — such as osteoblasts and osteoclasts. Molecules such as **parathyroid hormone-related protein** and transforming growth factor- β (TGF- β) that are produced by the cancer cells or are present in the bone microenvironment might mediate this growth⁴². Conversely, other types of cancer cell that happened to be taken by the circulation to the bone might not be similarly stimulated to grow — and would therefore remain undetected and clinically irrelevant. For example, Yoneda *et al.*⁴³ selected MDA-MB-231 breast cancer cells for increased ability to grow in bone after intracardiac injection, and found that the *in vitro* growth of the bone-selected cells — but not the parental cells — was promoted by **insulin-like growth-factor 1** and was not inhibited by TGF- β .

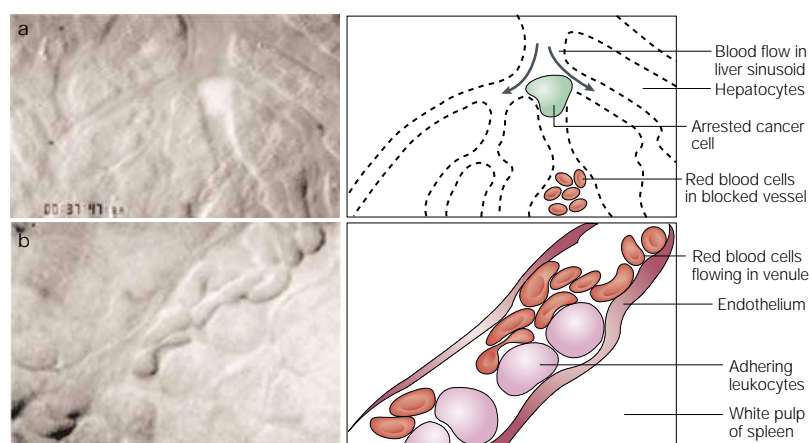


Figure 3 | Cancer and normal cells arrest in the circulation. **a** | *In vivo* videomicroscopic image of a mammary carcinoma cell arrested in mouse liver sinusoid by size restriction in a small tapering sinusoid of the liver, following injection of cells via a mesenteric vein to target them to the liver microcirculation. **b** | *In vivo* videomicroscopic image of white blood cells adhering to the endothelial wall of a venule in murine spleen — a vessel with a diameter that is larger than the cells — with blood flow continuing past the cells. The endothelial walls can clearly be seen. In non-cytokine treated mice, cancer cells arrest in organs by size restriction in small capillaries, or liver sinusoids as seen in **a**, and do not arrest in vessels larger than their own diameter, as do white blood cells (**b**). Schematics of the *in vivo* videomicroscopic images are shown on the right.

Some molecular factors that influence the ability of colon and other cancer cells to grow in the liver have also been identified. These include the expression of specific growth-factor receptors on the cancer cells — for example, the **epidermal growth-factor receptor** — coupled with expression of growth factors in the tissue, such as transforming growth factor- α (TGF- α)^{38–41}. In a study in which colorectal cancer cells of differing metastatic ability were implanted directly into liver, it was found that growth regulation in the liver environment determined the metastatic phenotype, although the molecular basis for this growth regulation remains to be determined⁴⁴.

The organ microenvironment can markedly change the gene-expression patterns of cancer cells, and therefore their behaviour and growth ability. For example, the same cancer cells, grown experimentally in two different sites, can express different levels of various proteolytic enzymes^{45,46}. Cancer cells that are present in different organ microenvironments can also be differentially responsive to chemotherapy⁴⁷. Research by Dalton⁴⁸ has identified soluble cytokines, such as **IL-6**, as well as cell-adhesion molecules, as contributors to microenvironment-mediated responses to chemotherapy and therapy-induced apoptosis. Furthermore, cancer cells might be differentially dependent on angiogenesis to support their growth, and differentially sensitive to anti-angiogenic therapy, depending on their p53 status⁴⁹. In addition, ISCHAEMIA in the organ microenvironment has been shown to induce VASCULAR MIMICRY, which could lead to enhanced cancer-cell survival and decreased dependence on the induction of host-derived neovasculature⁵⁰.

Recent studies on metastasis suppressor genes — which suppress the ability of metastases to form, but do

not alter tumorigenic ability at a primary site — are consistent with the idea that growth regulation in secondary sites is a crucial regulator of metastatic ability. So far, six such genes have been identified, and, although their mechanisms are poorly understood and molecularly diverse, their functional effect seems to be to regulate the ability of cells to initiate and maintain growth after the cells have arrived in a secondary site^{51–53}. Interestingly, the metastatic ability of mammary tumours in different mouse strains has been shown to be linked to allelic variations in one of these genes, the **BRMS1** metastasis suppressor gene⁵⁴. Regulation of cancer-cell growth ability in secondary sites has therefore been identified by this independent line of research as key to determining metastatic outcome.

Studies on the contribution of chemokine receptors to organ-specific metastasis are providing important clues about why some cancers metastasize to specific organs. Chemokines and their receptors are known to have a role in the ‘homing’ of lymphocytes and haematopoietic cells to specific organs^{55–57}. Recent elegant studies have shown that tumour cells express patterns of chemokine receptors that ‘match’ chemokines that are specifically expressed in organs to which these cancers commonly metastasize⁵⁸. For example, both breast cancer cells and primary breast tumours were found to express the chemokine receptors **CXCR4** and **CCR7** at high levels. The specific ligands for these receptors — **CXCL12** and **CCL21** — are found at elevated levels in lymph nodes, lung, liver and bone marrow — organs to which breast tumours often metastasize. Furthermore, blocking CXCR4 was found to inhibit metastasis of breast cancer cells in experimental animal models⁵⁸.

Because chemokines are involved in the ‘homing’ of lymphocytes, it was reasonable to suppose that chemokines also caused cancer cells to ‘home’ to specific secondary sites, thereby promoting organ-specific metastasis^{58–61}. However, this idea is difficult to resolve with the physical factors that seem to determine where circulating cancer cells arrest, as most circulating cells do not have the physical opportunity to recirculate and arrest by specific adhesive interactions. This apparent discrepancy is easily resolved when it is realized that chemokine–receptor interactions can initiate signalling pathways that lead to diverse cellular functions such as **actin** polymerization, invasion and the formation of pseudopodial projections, as well as activation of the **RAS/MAPK** (mitogen-activated protein kinase) pathways^{57,58,62}. So, by placing information about molecular factors that are shown to affect metastatic end points in the context of the physical and mechanical factors that affect the metastatic process, a better understanding of the way in which these molecular factors might function in metastasis will be obtained (FIG. 4). This, in turn, will aid the development of molecularly targeted anti-metastatic treatments.

RAS signalling pathways have long been known to affect metastatic outcome^{8,63–67}. Numerous molecular consequences of RAS signalling have been identified,

ISCHAEMIA

A reduction in local tissue oxygen levels due to inadequate blood supply.

VASCULAR MIMICRY

The formation of blood-flow channels that lack an endothelium, which might be formed by tumour cells in some tumours.

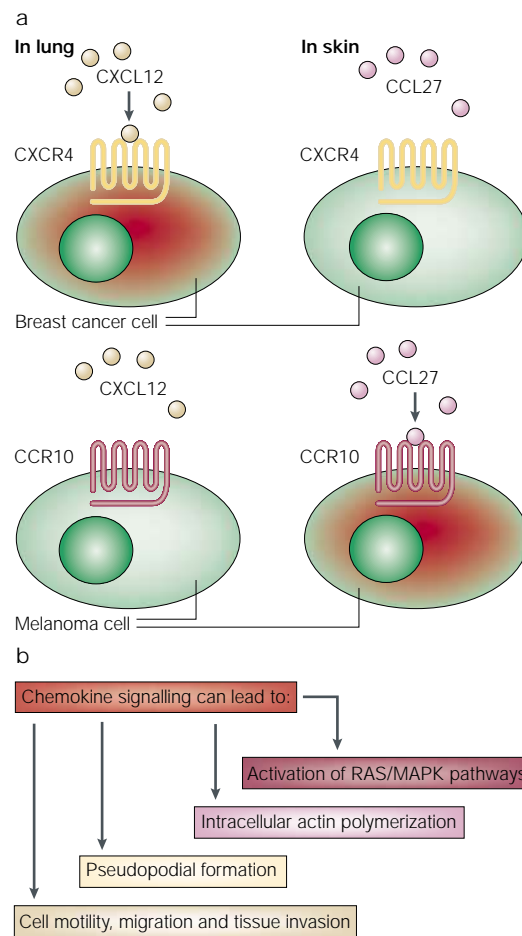


Figure 4 | Chemokines can influence organ-specific metastatic growth of cancer cells. **a** | Breast cancer cells express high levels of the CXCR4 chemokine receptor, whereas melanoma cells express high levels of the CCR10 receptor (and lower levels of CXCR4, not shown). Lung tissue expresses high levels of CXCL12, a soluble ligand for the CXCR4 receptor, whereas skin tissue expresses high levels of CCL27, a soluble ligand for the CCR10 receptor. Therefore, breast cancer cells that are taken to the lung by the blood flow would find a strong chemokine–receptor ‘match’, which would lead to chemokine-mediated signal activation. By contrast, breast cancer cells taken to skin would not find such a match. Melanoma cells, however, taken to skin by the circulation (or by local invasion) would find a CCL27–CCR10 chemokine–receptor ‘match’ that would lead to the activation of chemokine-mediated pathways. **b** | Activation of chemokine signalling can result in several changes in cells, including activation of RAS/MAPK (mitogen-activated protein kinase) signalling pathways, polymerization of intracellular actin and cytoskeletal changes, formation of pseudopodia, and increased cell motility, migration and tissue invasion. Any of these changes could contribute to the ability of cancer cells to survive and to initiate and maintain metastatic growth, and could therefore contribute to the regulation of organ-specific metastatic growth. Information on the tumour and tissue specificity of chemokine–ligand and –receptor expression is as presented in REF. 58, and information on chemokine signalling is as presented in REFS 57,58 and 62.

and much has been learned about the molecular details of RAS signalling (see REFS 68–76). RAS genes are activated by mutation in a large percentage of

human cancers⁷⁷, and the RAS pathways are activated in many other tumours through other mechanisms⁷⁸. However, the steps in metastasis that are affected by RAS activation have not been identified. Recent studies have shown that RAS-transformed and control NIH-3T3 fibroblasts extravasate equally well in experimental animal models^{32,79}. In a liver metastasis model, both cell types were also shown to initiate micrometastatic growth from a subset of cells that arrested in the liver³². However, only the micrometastases that formed from cells with activated RAS were able to maintain metastatic growth — the micrometastases formed from the control cells disappeared. In that study, it was found that the balance between apoptosis and proliferation was tipped in favour of growth in the cells with activated RAS signalling, and in favour of death in the control micrometastases (FIG. 5). Therefore, an activated RAS pathway was shown in this experimental model to support metastasis through regulation of the growth ability of the cells after they had arrived at the secondary site. Activation of RAS signalling by means of other routes (for example, through growth-factor or chemokine signalling) might also have similar effects on the proliferation:apoptosis balance — and therefore growth — of cells that arrive in a secondary organ, indicating one mechanism by which oncogenic signalling might affect growth (or growth failure) of cells that find themselves in a new organ.

The ability of cancer cells to grow in a specific site therefore depends on features that are inherent to the cancer cell, features inherent to the organ and the active interplay between these factors. Much remains to be learned about the detailed molecular interactions between specific cancer cells and specific secondary sites, and many organ-specific growth-factor pathways probably remain to be identified. But there is strong evidence to indicate that these interactions are important in determining whether a cell that arrives in an organ — or at a particular site within an organ³¹ — has a high or low probability of growing.

Metastasis is an inefficient process. It has been recognized for many years that metastasis is inherently an inefficient process^{80,81}, but which particular steps are inefficient? Millions of cancer cells can be injected into an experimental mouse, and these might give rise to only a few metastases that are detectable in ‘end-point’ assays. Similarly, large numbers of cancer cells might be detected in the blood in cancer patients, and yet very few of these develop into overt metastases. What, then, is the fate of most cancer cells that enter the circulation?

This question has been addressed using high-resolution *in vivo* video microscopy and quantitative cell-fate analyses that monitor the loss of cells over time during metastasis^{4,14,18,30–32}. These studies have led to the conclusion that early steps in the haematogenous metastatic process — from the time that cancer cells enter the bloodstream until they extravasate into secondary organs — are completed remarkably efficiently, at least in the

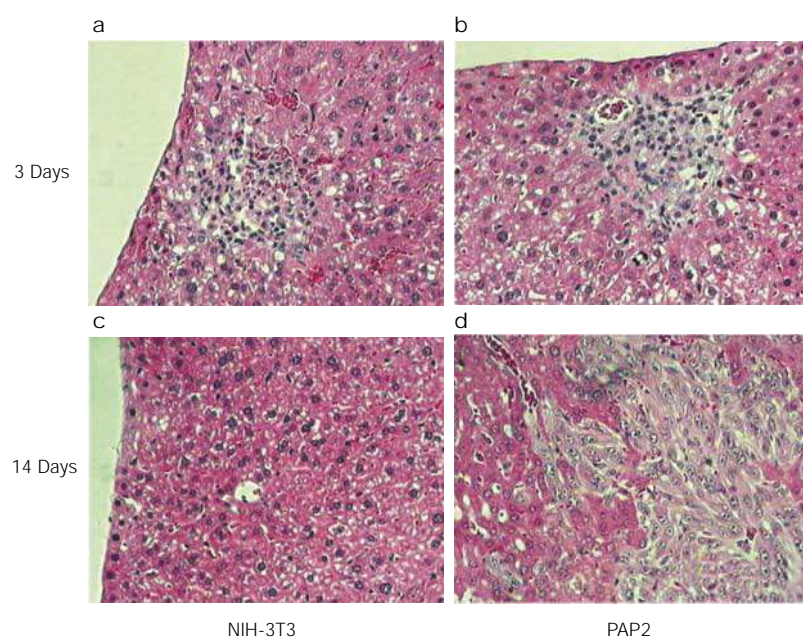


Figure 5 | Activation of RAS signalling pathways can protect small metastases and promote their early growth. When metastatic *HRAS*-transformed (PAP2) and non-tumorigenic control NIH-3T3 fibroblasts were injected into mesenteric veins of mice to target them to the liver, both cell lines initiated growth of micrometastases (panels **a** and **b**, 3 days after injection), as seen in histological sections. However, only the micrometastases that were formed from the *RAS*-transformed PAP2 cell line persisted to form macroscopic metastases (panel **d**), whereas the NIH-3T3 micrometastases disappeared (panel **c**). In the *RAS*-expressing PAP2 micrometastases, the balance between apoptosis and proliferation was tipped to favour progressive growth, whereas in the NIH-3T3 micrometastases, this balance was tipped to favour apoptosis and disappearance of early metastases. Reprinted with permission from REF. 32 © (2002) American Association for Cancer Research.

experimental models studied. By contrast, subsequent steps in the metastatic process are completed inefficiently, with only a small subset of cancer cells in a secondary site initiating cell division to form micrometastases, and only a small proportion of these micrometastases persisting to become vascularized and progressively growing macroscopic metastases.

For example, the fate of B16F1 murine melanoma cells — after their injection into mice through the mesenteric vein, to target the cells to the liver — was followed over time using quantitative cell-fate analysis³⁰. (This technique uses 10 µm inert plastic microbeads, co-injected with the cancer cells in a defined ratio — for example, one bead for every ten cancer cells. The beads are carried to the target organ along with the cells and arrest there, providing a permanent reference to quantify any cell loss at later times¹⁸.) It was found that over 87% of the injected cells were arrested in the liver and were present there 90 minutes after injection³⁰. Whether the remaining 13% of cells were killed or passed through the organ could not be determined in that study, but other studies have indicated that apoptosis might contribute to early loss of cancer cells in a new organ⁸², which is consistent with the idea that very few cells pass through the first capillary bed that they encounter. Three days after injection, 83% of the cells that were originally injected had extravasated into the

liver parenchyma and remained there, but only a very small proportion of them (2% of the injected cells) had formed micrometastases³⁰. Furthermore, not all of these micrometastases persisted, and the progressively growing metastases that would have killed the mice arose from a tiny subset (only 0.02%) of the injected cells.

So, the initial steps in haematogenous metastasis — arrest in the organ and extravasation — were performed with very high efficiency (FIG. 1a). Subsequent steps — the initiation of growth to form micrometastases and persistence to form macroscopic metastases — were considerably less efficient (FIG. 1b). Regulation of growth of a subpopulation of cells that arrested in an organ was therefore responsible for the overall metastatic inefficiency. Similar conclusions have been obtained for mouse B16F10 melanoma cells that were injected to target the lung³¹, and for *RAS*-transformed and control mouse fibroblasts³² and mouse mammary carcinoma cells⁸³ that were injected to target them to the liver. In all cases, the initial arrest of cells was very efficient, but the initiation and persistence of growth was much less efficient. In the case of B16F10 cells that were injected to target them to the lung, 98% of the injected cells were arrested in the lung and found there 1 hour after injection³¹, indicating that very few cells were able to pass through the lung and go to other organs. Metastatic inefficiency, as reflected in the end point of visible metastases, would therefore seem to be due primarily to the regulation of cancer-cell growth in secondary sites.

Metastases can arise long after the apparently successful treatment of a primary tumour. In breast cancer or melanoma, for example, metastases have been known to occur decades after primary treatment^{84,85}. Where are the cancer cells during this period of dormancy, and what awakens them? These questions are clinically important, but are largely unanswered at present^{86,87}. Studies that have modelled dormancy mathematically indicate that continuous slow growth is unlikely, favouring instead a model of discontinuous growth and periods of quiescence^{88,89}.

Like the metastatic process itself, dormancy is a 'hidden' state, and is difficult to observe and study directly. Experimental studies, however, are now shedding some light on tumour dormancy. Judah Folkman and his colleagues have provided evidence for the existence of pre-angiogenic micrometastases, in which cells actively divide, but at a rate that is balanced by the apoptotic rate because of failure of the micrometastases to become vascularized^{90,91}. Pre-angiogenic micrometastases could therefore be one source of tumour dormancy. If these small metastases subsequently acquire the ability to become vascularized, dormancy might cease and tumour growth would occur.

Another possible contributor to tumour dormancy is the persistence of solitary cells in secondary sites^{30–32,83,87,92,93}. Large numbers of solitary cells, which arrive in a secondary site but fail to initiate cell division, can persist for long periods of time in the organ. Furthermore, these cells can persist in a background of actively growing metastases, indicating that both dormant and non-dormant cells can be present in a

TRASTUZUMAB

(Herceptin). A humanized monoclonal antibody against the ERBB2 receptor that is used to treat breast cancers that are shown to be positive for this receptor.

IMATINIB

(Glivec). A small-molecule therapy that targets the Abelson leukaemia (ABL) kinase. It is used to treat patients with chronic myelogenous leukaemia.

FARNESYLTRANSFERASE INHIBITORS

Therapeutic agents that target a specific post-translational modification — farnesylation — which allows the RAS protein to attach to the inner cell membrane and which is necessary for RAS-mediated signalling.

BISPHOSPHONATES

A class of compounds that inhibit the bone-resorptive activity of osteoclasts, and are used to treat osteoporosis. They might also be useful in the treatment and prevention of metastases growing in the bone.

secondary site (FIG. 1b). After recovery from liver tissue, solitary dormant mammary carcinoma cells retained their ability to form tumours when re-injected into mammary fat pads of mice⁸³. So, despite their apparent dormancy at a secondary site, the recovered cells still retained their tumorigenic phenotype. A better understanding of molecular factors that contribute to the maintenance and subsequent release from dormancy in secondary sites, both of solitary cells and pre-angiogenic metastases, will be important in treating this aspect of metastatic disease.

Therapeutic implications

How can the growing wealth of molecular knowledge about factors that influence the end point of metastasis best be used in the context of the physical and mechanical factors that affect cancer cells during the metastatic process? Which steps in the metastatic process are good targets for therapy? In theory, inhibition of any of the steps in the metastatic process — from the initial release of cells into the circulation at the site of the primary tumour, to the final stages of growth in the new organ — could offer therapeutic targets. In practice, however, two factors limit the potential success of targeting any phase of the metastatic process. First, is the step clinically accessible? Second, is the process a good biological target?

Clinically, by the time a primary tumour is detected, it might already have seeded tumour cells to secondary sites. So, targeting early steps in metastasis is less likely to be effective, as they might have already occurred at the time of diagnosis. Later steps might not have occurred at the time of cancer diagnosis, so would offer more promising targets for therapy. Moreover, targeting a very inefficient biological process might be easier than targeting an efficient one, because fewer cells would have to be inhibited.

Both of these factors indicate that the growth phase of the metastatic process is a promising therapeutic target. As shown in FIG. 1b, cancer cells can exist in three separate states in a secondary site: solitary cells that are not dividing; active pre-angiogenic

micrometastases in which proliferation is balanced by apoptosis with no net increase in tumour size; and vascularized metastases, which might be either quite small and clinically undetectable or larger and detectable by current technology. Furthermore, cells in all three states might be present in the same organ at the same time. Any therapy that prevents the progression of metastases to form clinically damaging tumours has the potential to benefit patients.

Current anti-angiogenic strategies clearly meet the requirements of being both clinically accessible and biologically relevant, and would target both the growth of vascularized metastases and the progression of micrometastases to a vascularized state. These growth stages would also be targeted by anti-growth therapies, such as cytotoxic chemotherapies and molecularly based strategies that are designed to block specific growth pathways (for example, TRASTUZUMAB (Herceptin), IMATINIB (Glivec) and FARNESYLTRANSFERASE INHIBITORS^{94–97}). Also included in this category would be BISPHOSPHONATE treatment to prevent the growth of metastases in bone^{98,99}, which represents a strategy that targets the ‘soil’ rather than the ‘seed.’ At this stage, however, little is known about how the initiation of the growth of solitary, non-dividing cells is induced or could be prevented, and this will require further study.

Metastatic growth therefore offers a temporally broad target, which is both biologically and clinically appropriate. Any therapy that prevents the growth of metastases and the subsequent physiological damage caused by this growth has potential clinical utility. The search for ‘anti-metastatic’ agents must be broadened to include organ-specific growth as an appropriate target. The ‘seed’ and ‘soil’ compatibility that leads to organ-specific growth promotion of certain types of cancers in specific organs is itself a therapeutic target, if ways to interfere with the dependence of the cancer cell on factors present in the target organ can be identified. To achieve this aim, a better understanding of the factors that influence organ-specific metastatic growth of individual tumour types in various target organs is needed.

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DATABASES

The following terms in this article are linked online to: **Cancer.gov:** http://www.cancer.gov/cancer_information/ bone cancer | brain cancer | breast cancer | colorectal cancer | liver cancer | lung cancer | melanoma | prostate cancer | stomach cancer | testicular cancer
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