TIMELINE

The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited

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Researchers have been studying metastasis for more than 100 years, and only recently have we gained insight into the mechanisms by which metastatic cells arise from primary tumours and the reasons that certain tumour types tend to metastasize to specific organs. Stephen Paget's 1889 proposal that metastasis depends on cross-talk between selected cancer cells (the 'seeds') and specific organ microenvironments (the 'soil') still holds forth today. It is now known that the potential of a tumour cell to metastasize depends on its interactions with the homeostatic factors that promote tumour-cell growth, survival, angiogenesis, invasion and metastasis. How has this field developed over the past century, and what major breakthroughs are most likely to lead to effective therapeutic approaches?

Metastasis — the spread of cells from the primary neoplasm to distant organs, and their relentless growth — is the most fearsome aspect of cancer. This fear is well founded. Despite significant improvements in diagnosis, surgical techniques, general patient care, and local and systemic adjuvant therapies, most deaths from cancer are due to metastases that are resistant to conventional therapies. The main barrier to the treatment of metastases is the biological heterogeneity of cancer cells in the primary neoplasm and in metastases. Furthermore, the specific organ microenvironment can modify the response of a metastatic tumour cell to systemic therapy. Continual empiricism in the treatment of cancer metastasis is unlikely to produce significant improvements in therapy. Therefore, understanding the pathogenesis of metastasis on the systemic, cellular and molecular levels are important goals of cancer research.

During the past 30 years, the study of cancer metastasis has grown exponentially. A thorough historical review of the field by the late Leonard Weiss has recently been published[1], so this article will cover only the evolution of research that deals with the cellular origin of cancer metastases and their interaction with the organ microenvironment.

The concept that metastasis results when tumour cells interact with a specific organ microenvironment is not new (see TIMELINE). In 1889, the English surgeon Stephen Paget (FIG. 1) published the seminal 'seed and soil' hypothesis to explain the non-random pattern of metastasis[2]. Paget was struck by the discrepancy between the relative blood supply and the frequency of metastases in certain organs. He scrutinized more than 900 autopsy records of patients with different primary tumours. His analysis documented a non-random pattern of metastasis to visceral organs and bones. These findings indicated to Paget that the outcome of metastasis was not due to chance (the prevailing viewpoint of that time), but that certain tumour cells (which he equated to the 'seed') have specific affinity for the milieu of certain organs (which he equated to the 'soil'). He concluded that metastases formed only when the seed and soil were compatible.

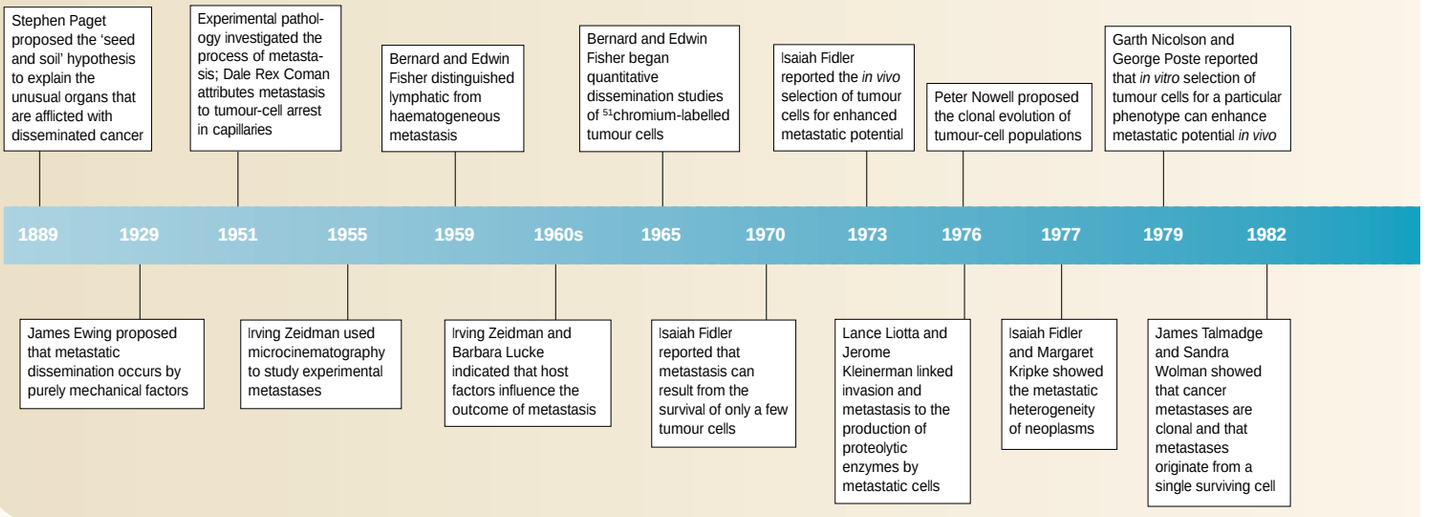
In 1929, James Ewing challenged Paget's 'seed and soil' theory, and proposed that metastatic dissemination occurs by purely mechanical factors that are a result of the anatomical structure of the vascular system[3]. Ewing's viewpoint prevailed for several decades. In the 1970s, the selective nature of metastasis was documented. A detailed analysis of experimental metastasis in syngeneic mice indicated that mechanical arrest of tumour cells in the capillary bed of distant organs could indeed occur, but that subsequent proliferation and growth into secondary lesions were influenced by specific organ cells[4]. Weiss[1] and Sugarbaker[5] reviewed clinical data on site preferences of metastases produced by different human neoplasms. They concluded that common regional metastatic involvement could be attributed to anatomical or mechanical considerations, such as efferent venous circulation or lymphatic drainage to regional lymph nodes, but that metastasis to distant organs from numerous types of cancers were indeed site specific.

In 1989, a symposium commemorated the centennial anniversary of Paget's 'seed and soil' hypothesis. In his introductory remarks to the 1989 symposium, George Poste commented, "There are few scientists, historical or contemporary, whose work will withstand 100 years of scrutiny and not succumb to the depressing trend of modern publications — to ignore papers published more than five years ago". Hopefully, this article will provide an interesting perspective on Paget's magnificent contribution.

The pathogenesis of a metastasis

The process of cancer metastasis consists of a long series of sequential, interrelated steps. Each of these can be rate limiting, as a failure or an insufficiency at any of the steps can stop the entire process[6,7]. The outcome

Timeline | Retrospective analysis of the pathogenesis of cancer metastasis



of the process is dependent on both the intrinsic properties of the tumour cells and the responses of the host (FIG. 2; TABLE 1).

The age of experimental pathology. In the 1950s, the Department of Pathology at the University of Pennsylvania School of Medicine was unique in that several of its faculty studied cancer invasion and metastasis. The Chairman, Dale Rex Coman, had recruited many outstanding pathologists, including Irving Zeidman, Charles Breedis, Gabriel Gasic and Peter Nowell. This group of investigators was largely responsible for advancing the research on experimental pathology, and generated the interest in elucidating some of the factors that are responsible for the unusual distribution of bloodborne metastases in humans and experimental animals.

Figure 1 | **Stephen Paget.**

In 1951, Coman *et al.*[8] reported that the direct intravascular injection of tumour cells into animals produced metastases in some, but not all, visceral organs. The authors found that in those organs, circulating tumour cells were lodged in the capillaries, whereas in organs that were rare sites of metastasis, circulating cells lodged in arterioles. This observation indicated that the distribution of metastases was largely dependent on mechanical factors — that is, on the arrest of emboli in capillaries of secondary organs. In 1952, Lucke *et al.*[9] compared carcinoma metastases in the livers and lungs of rabbits, and found that liver metastases were larger and more numerous. Human cancer patients also develop a larger number of liver metastases than of lung metastases, so both mechanical and local 'soil' factors are likely to determine whether or not a metastasis will develop after the arrest of tumour emboli.

Some quantitative studies of various phases of metastasis had been completed by the early 1950s. My mentor, Irving Zeidman, reported in 1950 that the number of metastases that develop is directly proportional to the number of tumour cells injected intravenously, but that most injected tumour cells still fail to form tumours[10]. A decade later, Zeidman and colleagues used CINEPHOTOMICROGRAPHY to observe the incidence of emboli arrest in mesenteric capillaries of rabbits. They found that some tumour cells became distorted and passed through the narrow capillary tube, whereas others appeared more rigid and were trapped. The incidence of arrest varied with the type of tumour studied. This work established the morphological foundation for previous indirect demonstrations that some tumour-cell emboli could pass immediately through the vascular bed of organs[11]. These experiments yielded qualitative information on the fate of circulating cancer cells. Some cells pass through the narrow vessels of an organ immediately. Of those cells arrested, some yield metastases, whereas others die.

The search for quantitative information dealing with tumour-cell arrest and dissemination was considerably advanced with the

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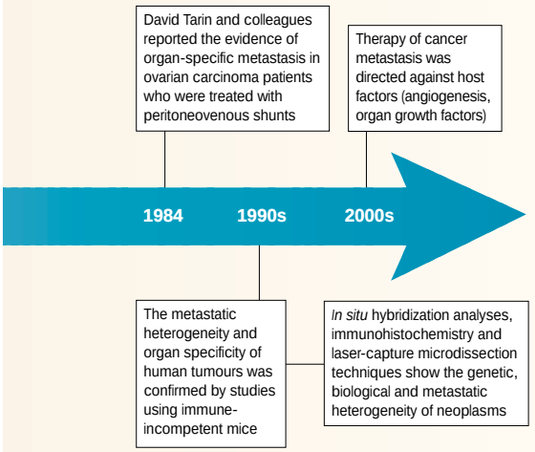
(Stephen Paget)



advent of radioactive labelling of tumour cells. A thorough review of the literature on this topic, however, could have been accomplished in one night[12,13]. Bernard and Edwin Fisher reported that they could quantify metastasis by tracking tumour cells that were labelled with ^{51}chromium. The results, however, were controversial, as ^{51}chromium was released by dead cells and re-internalized by living cells[14].

To overcome this deficiency, researchers began to label cancer cells with ^{125}iodine-iodo-deoxyuridine, which is incorporated into the DNA and is therefore not released from cells. These studies showed that within 24 hours after entry into the circulation, less than 0.1% of tumour cells are still viable, and that less than 0.01% of these cells, when introduced into the circulation, survive to produce metastases[15]. Therefore, only a few cells in a primary tumour can give rise to a metastasis[16]. This prompted the question of whether the development of metastases represents the fortuitous survival and growth of very few neoplastic cells, or whether it represents the selective growth of unique subpopulations of malignant cells that are endowed with special properties. Subsequent studies clearly showed that neoplasms are biologically heterogeneous and that the process of metastasis is selective.

Biological heterogeneity in neoplasms

At the time of diagnosis, many human and animal tumours are heterogeneous and contain numerous subpopulations of cells that have different biological characteristics, including metastatic potential[17–19]. This biological diversity can result from a tumour's multicellular origin, but in tumours that originate from a single transformed cell, the source of the biological diversity is less clear.

Clinical and experimental studies have shown that neoplasms undergo a series of changes during the course of the disease. For example, a growth that is initially benign can change into a malignant, lethal tumour. Leslie Foulds described this phenomenon of tumour evolution as 'neoplastic progression', and defined it as "acquisition of permanent, irreversible qualitative changes in one or more characteristics of a neoplasm"[20]. This evolution of tumours is gradual, and tumour cells proceed towards increased autonomy from their host by a temporal change in various independent characteristics. Moreover, because tumour progression can occur over periods of years, the behaviour of a neoplasm can vary at different disease stages.

This evolution has been attributed to acquired genetic variations in the cells that populate a neoplasm[21]. Peter Nowell predicted that those tumour cells that progress to an advanced stage of malignancy — that is, metastatic cells — would be less stable, genetically, than non-metastatic tumour cells. This hypothesis was tested using the Luria and Delbruck fluctuation analysis[22]. This analysis is based on acquisition of mutations that confer resistance to the metabolite 6-thiopurine (caused by a mutation in hypoxanthine/guanine phosphoribosyl-transferase) and resistance to the drug ouabain (caused by a mutation in the

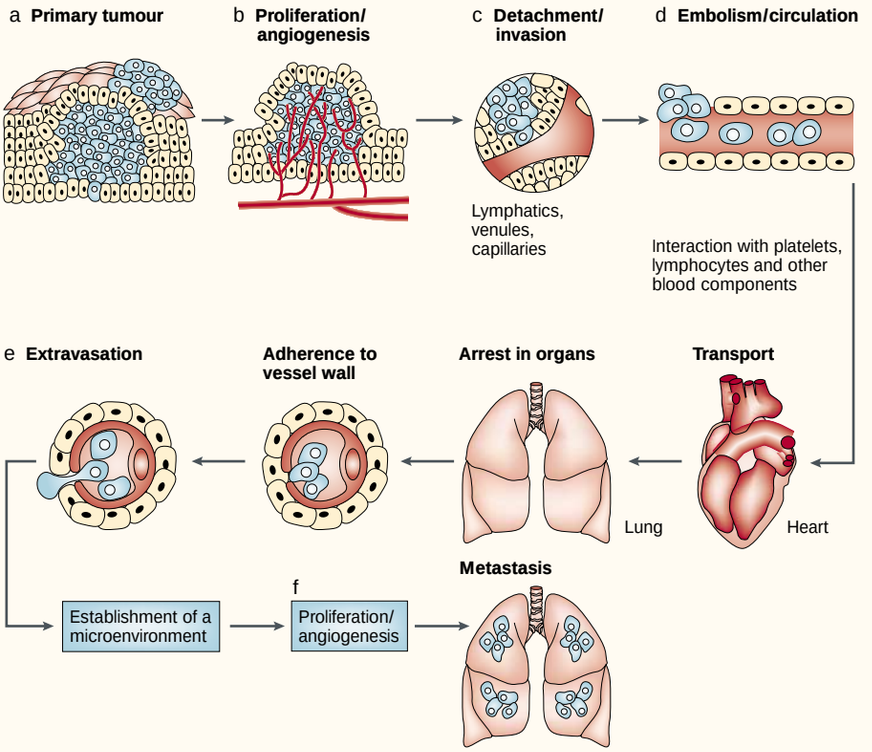


Figure 2 | **The main steps in the formation of a metastasis. a** | Cellular transformation and tumour growth. Growth of neoplastic cells must be progressive, with nutrients for the expanding tumour mass initially supplied by simple diffusion. **b** | Extensive vascularization must occur if a tumour mass is to exceed 1–2 mm in diameter[39]. The synthesis and secretion of angiogenic factors establish a capillary network from the surrounding host tissue[39]. **c** | Local invasion of the host stroma by some tumour cells occurs by several parallel mechanisms[40]. Thin-walled venules, such as lymphatic channels, offer very little resistance to penetration by tumour cells and provide the most common route for tumour-cell entry into the circulation[12,41]. **d** | Detachment and embolization of single tumour cells or aggregates occurs next, most circulating tumour cells being rapidly destroyed. After the tumour cells have survived the circulation, they become trapped in the capillary beds of distant organs by adhering either to capillary endothelial cells or to subendothelial basement membrane that might be exposed[27]. **e** | Extravasation occurs next — probably by mechanisms similar to those that operate during invasion. **f** | Proliferation within the organ parenchyma completes the metastatic process. To continue growing, the micrometastasis must develop a vascular network[39] and evade destruction by host defences. The cells can then invade blood vessels, enter the circulation and produce additional metastases[6,7].

Table 1 | Regulation of metastasis

Cell type	Facilitation of metastasis	Inhibition of metastasis
Tumour cells	Production of growth factors and their receptors	Antigenicity
	Production of angiogenic factors	Inhibitors of angiogenesis
	Motility, invasiveness	Cohesion (E-cadherin)
	Aggregation, deformability	Tissue inhibitors of proteolytic enzymes
	Specific cell-surface receptors and adhesion molecules	
Host cells	Paracrine and endocrine growth factors	Tissue barriers
	Neovascularization	Blood turbulence, endothelial cells
	Platelets and their products	Tissue inhibitors of proteolytic enzymes
	Immune cells and their products	Antiproliferative factors
		Inhibitors of angiogenesis

cell-membrane-bound Na^+, K^+-ATPase)[23]. The development of drug resistance was compared between highly metastatic cells from three different mouse tumours, cells with low metastatic potential and non-metastatic tumour cells isolated from the same neoplasms. In all cases, cells with high metastatic potential had a three- to sevenfold increase in the rate of mutation (per cell generation) at both genetic loci, compared with their low metastatic but tumorigenic cell controls[23]. These results support the hypothesis that the evolution of tumours from the benign to the malignant state could be the consequence of acquired genetic instability[24].

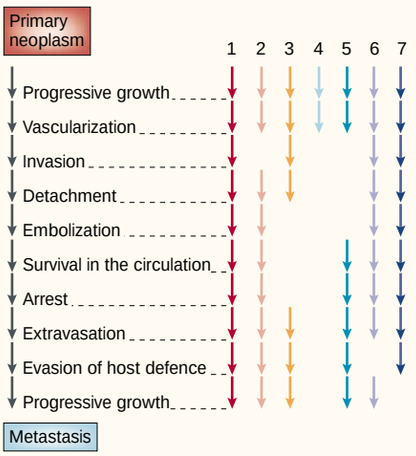


Figure 3 | **Sequential steps in the pathogenesis of cancer metastasis.** Each discrete step of metastasis (arrows) is likely to be regulated by transient or permanent changes in DNA, RNA or proteins. Most cancer cells fail to undergo metastasis because of one or more deficiencies (gaps between arrows). Metastasis-competent cells (1) must perform a variety of tasks. Cancer-cell metastasis can be blocked at a variety of stages, including deficiencies in invasion, or survival and proliferation in the circulation (2, 3); or multiple deficiencies that prohibit metastasis (4 and 5). Other tumour-cell phenotypes that preclude metastasis include susceptibility to immune-system attack (6) and an inability to grow at the final metastatic site (7).

Metastatic heterogeneity

Cells with different metastatic properties have been isolated from the same parent tumour, indicating that not all the cells in a primary tumour have the same potential to disseminate (FIG. 3). To study this, tumour cells are implanted subcutaneously, intramuscularly, directly into tissues or are injected intravenously into mice. Tumours are then harvested, and the recovered cells are expanded in culture. The behaviour of the expanded cells is compared to that of the cells of the parent tumour to determine whether the selection process enhanced metastatic capacity. This procedure was originally used to isolate the B16-F10 line from B16 melanoma[25]. It has also been successfully used to derive metastatic cell lines from many commonly studied experimental tumours[26].

In a second approach, cells are selected for the development of a phenotype that is associated with the metastatic sequence, and then they are tested in animal models to determine whether concomitant metastatic potential is increased or decreased. This method has been used by Nicolson[27] and Poste *et al.*[26] to determine whether properties such as adhesive characteristics, invasive capacity, lectin resistance and resistance to natural-killer cells are required for metastasis.

One obvious criticism of these approaches has been that the cancer cells studied do not represent the progeny of a unique subpopulation of tumour cells; they are the progeny of tumour cells that can survive in a new microenvironment. The first experimental proof for metastatic heterogeneity of neoplasms was provided by Fidler and Kripke in 1977, from work with the mouse B16 melanoma[28]. Using a modified fluctuation assay of Luria and Delbruck[22], they showed that different tumour-cell clones, each derived from individual cells isolated from a parent tumour, vary markedly in their ability to form pulmonary nodules following intravenous inoculation into syngeneic mice. Controlled subcloning procedures showed that the observed diversity was not a consequence of the cloning procedure[28] (FIG. 4).

To exclude the possibility that the metastatic heterogeneity of B16 melanoma cells might have been introduced as a result of the lengthy cultivation, researchers studied the biological and metastatic heterogeneity of spontaneous tumours. Melanomas were induced in mice by chronic exposure to ultraviolet-B irradiation and the tumour-promoting agent CROTON OIL. Tumour metastases were found to differ greatly from each other and from the parent tumour. In addition to differences in the number of metastases that developed from each tumour, there was also significant variability in the size and pigmentation of the metastases. Metastases to the lymph nodes, brain, heart, liver and skin were found in addition to lung metastases — those growing in the brain were uniformly pigmented, whereas those growing in other organs generally were not[29].

The finding that cell subpopulations that pre-exist within the same tumour have heterogeneous metastatic potential has since been confirmed in many laboratories, in a wide range of tumours of different histological origin. In addition, studies that involved young nude mice as models for metastasis of human neoplasms have shown that several human tumour lines and freshly isolated tumours, such as colon carcinoma and renal-cell carcinoma, also contain subpopulations of cells with widely differing metastatic properties[30].

The clonal origin of metastases

Biological heterogeneity is found both within a single metastasis (intralesional heterogeneity) and among different metastases (interlesional heterogeneity). This heterogeneity reflects two main processes. These include the selective nature of the metastatic process and the rapid evolution and phenotypic diversification of clonal tumour growth, which results from the inherent genetic and phenotypic instability of many clonal populations of tumour cells. Like primary neoplasms, metastases can have a unicellular or multicellular origin. To determine whether

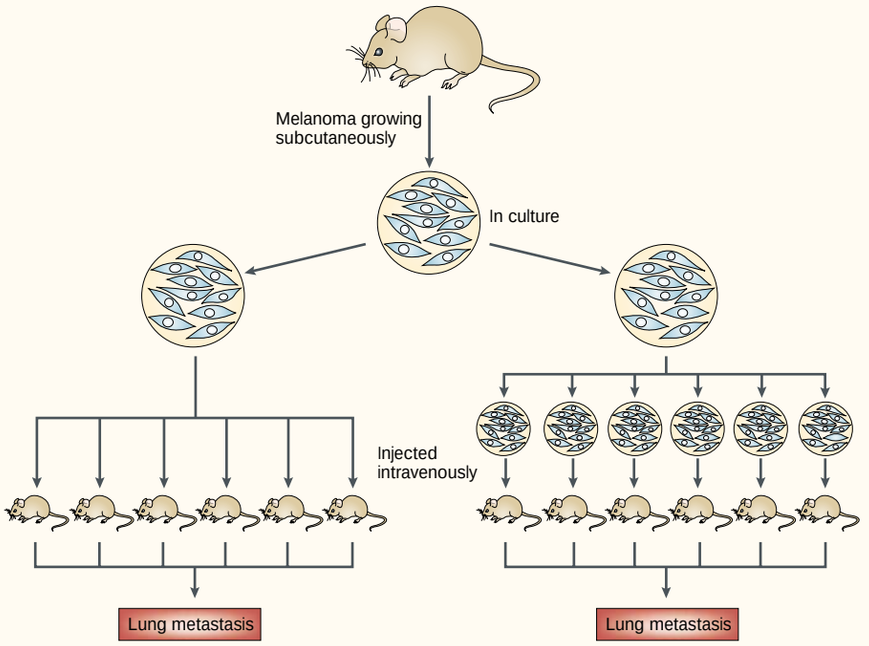


Figure 4 | **Metastatic heterogeneity.** A heterogeneous parental neoplasm is grown in culture and divided into two aliquots. One culture is cloned (right side of figure). Cells from unselected parental tumour (left panel) and cells from individual clones (right panel) are injected into syngeneic mice. Production of metastasis is determined several weeks later. These studies showed that different tumour-cell clones vary significantly in their ability to form pulmonary nodules following intravenous inoculation into syngeneic mice. The observed diversity was not a consequence of the cloning procedure[28].

all metastases arise from the same clone, or whether different metastases are produced from different progenitor cells, Talmadge *et al.* designed a series of experiments based on the fact that gamma-irradiation of tumour cells induces random chromosome breaks and rearrangements that serve as 'markers'. They examined spontaneous lung metastases that arose from subcutaneously implanted K-1735 mouse melanoma cells that had been gamma-irradiated to induce chromosomal damage[31]. In ten metastases, all the chromosomes were normal, making it impossible to establish whether they were of uni- or multicellular origin. In many other lesions, unique karyotypic patterns of abnormal marker chromosomes were found, indicating that each metastasis originated from a single progenitor cell. Subsequent experiments showed that when heterogeneous clumps of two different melanoma cell lines were injected intravenously, resultant lung metastases were all of unicellular origin[32]. These results indicated that whether an embolus is homogeneous or heterogeneous, metastases still originate from a single proliferating cell. Clonality of metastases has also been reported for many other tumours, including breast cancer and fibrosarcoma, as well as melanoma.

Glossary

ASCITES
The intraperitoneal accumulation of transudate (watery fluid).

CINEPHOTOMICROGRAPHY
The process of recording (making movies of) the movements of objects that are viewed through a microsope.

CROTON OIL
Oil that is produced from the seed of the tree *Croton tiglium*. It is an irritant that is used as a tumour promoter.

PERITONEOVENOUS SHUNTS
Drainage of ascitic fluids into the jugular vein through a tube.

The organ microenvironment

Clinical observations of cancer patients and studies in rodent models of cancer have revealed that certain tumour types tend to mestatasize to specific organs, independently of vascular anatomy, rate of blood flow and the number of tumour cells delivered to each organ. Experimental data to support Paget's 'seed and soil' hypothesis were derived from studies on the preferential invasion and growth of B16 melanoma metastases in specific organs. When mouse melanoma cells were introduced into the circulation of syngeneic mice, tumour growths developed in the lungs and in fragments of pulmonary or ovarian tissue that were implanted intramuscularly. By contrast, metastatic lesions did not develop in implanted renal tissue, or at the site of surgical trauma. This indicates that sites of metastasis are determined not solely by the characteristics of the neoplastic cells but also by the microenvironment of the host tissue[4].

Ethical considerations rule out the experimental analysis of cancer metastasis in patients, but the introduction of PERITONEOVENOUS SHUNTS for the palliation of ASCITES in women with progressive ovarian cancer has provided the opportunity to study some of the factors that affect metastatic spread in humans. Human ovarian cancer cells can grow in the peritoneal cavity, either in the ascites fluid or by attaching to the surface of peritoneal organs. These malignant cells, however, do not metastasize to other visceral organs. One (incorrect) explanation for the lack of visceral metastases was that the tumour cells could not gain entrance into the systemic circulation. David Tarin and colleagues studied metastasis in ovarian cancer patients whose ascites were drained into the venous circulation[33,34]. Although palliation and minimal complications were reported in all patients, the procedure allowed the entry of viable cancer cells into the jugular vein. The autopsy findings from 15 patients substantiated the clinical observations that the shunts did not significantly increase the risk of metastasis to organs outside the peritoneal cavity. In fact, despite continuous entry of millions of tumour cells into the circulation, metastases to the lung — the first capillary bed encountered — were rare[33,34]. These results provided compelling verification of the venerable 'seed and soil' hypothesis.

An interesting demonstration of organ-specific metastasis has come from studies of cerebral metastasis after injection of syngeneic tumour cells into the internal carotid artery of mice. Remarkable differences between two mouse melanomas were found in the patterns of brain metastasis — the K-1735 melanoma produced lesions only in the brain parenchyma, whereas the B16 melanoma produced only meningeal growths[35]. So, an explanation for the different sites of tumour growth within one organ could be based on interactions between the metastatic cells and the organ environment — possibly in terms of specific binding to endothelial cells and responses to local growth factors.

Seed and soil 2003

Since the 1980s, many investigators have made valuable contributions to our understanding of cancer pathogenesis and metastasis, and the dependency of these processes on the interaction between cancer cells and homeostatic factors[1,7]. A current definition of the 'seed and soil' hypothesis consists of three principles. First, primary neoplasms (and metastases) consist of both tumour cells and host cells. Host cells include epithelial cells, fibroblasts, endothelial cells and infiltrating leukocytes. Moreover, neoplasms are biologically heterogeneous and contain genotypically and phenotypically diverse subpopulations of tumour cells, each of which have the potential to complete some steps in the metastatic process, but not all. Recent studies involving *in situ* hybridization and immunohistochemical staining have shown that the expression of genes/proteins associated with proliferation, angiogenesis, cohesion, motility and invasion vary among different regions of neoplasms[7,36]. So, the search for genes/proteins that are associated with metastasis cannot be conducted by the indiscriminate and non-selective processing of tumour tissues. The recent advent of laser-capture microdissection, which allows for the isolation of specific tumour cells[36], provides an appropriate method to analyse tumour-cell heterogeneity.

Second, the process of metastasis is selective for cells that succeed in invasion, embolization, survival in the circulation, arrest in a distant capillary bed, and extravasation into and multiplication within the organ parenchyma (FIG. 3). The successful metastatic cell (the 'seed') has been likened to a decathlon champion who must be proficient in all ten events, rather than just a few[17]. Although some of the steps in this process contain stochastic elements, as a whole metastasis favours the survival and growth of a few subpopulations of cells that pre-exist within the parent neoplasm. So, metastases can have a clonal origin, and different metastases can originate from the proliferation of different single cells[17,32].

Third, and perhaps the most important principle for the design of new cancer therapies, is that metastases can only develop in specific organs. The microenvironments of different organs (the 'soil') are biologically unique. Endothelial cells in the vasculature of different organs express different cell-surface receptors[37] and growth factors that influence the phenotype of metastases that develop there[38]. In other words, the outcome of metastasis depends on multiple interactions ('cross-talk') of metastasizing cells with homeostatic mechanisms, which the tumour cells can usurp. Therapy of metastases, therefore, should be targeted not only against the cancer cells themselves, but also against the homeostatic factors that promote tumour-cell growth, survival, angiogenesis, invasion and metastasis.

Perhaps it is best to end this discussion with the last few sentences of the seminal publication by Paget: "The best work in the pathology of cancer now is done by those who, like Mr Balance and Mr Shattock, are studying the nature of the seed. They are like scientific botanists, and he who turns over the records of cases of cancer is only a ploughman, but his observations of the properties of the soil might also be useful"[2]. Indeed.

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doi: 10.1038/nrc1098

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