The Biology of Cancer Metastasis

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CONTENTS

KEY POINTS

- For at least one century, the prevailing view has considered metastasis as a late and final step in cancer progression. Also, supportive experimental data have been gathered, such as somatic genetic changes accumulating during local cancer progression—many of which can also be identified in metastases.
- More recent genetic data suggested that the metastatic potential cannot be acquired late in local progression in rare variant cells, but that dissemination of tumour cells begins very early after transformation. Primary tumours may often be poor surrogate markers for the genetics of disseminated tumour cells (DTCs) and thereby for response to adjuvant therapies.
- The cancer stem cell (CSC) hypothesis adds as a further complication a hierarchy of tumour cells, generated by non-genetic mechanisms, to the progression puzzle. This hypothesis assumes that only rare subpopulations of tumour cells, derived from organ-specific stem or progenitor cells, are driving the growth and spread of malignant cancers.
- Cytokeratins are the most specific and currently also the most sensitive markers to detect single DTCs in bone marrow, while the epithelial cell adhesion molecule (EpCAM; CD326) is favoured for the analysis of DTCs in lymph nodes, and for the detection of circulating tumour cells (CTC) in the blood stream several markers are in use.

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- Several lines of evidence suggest that DTCs evolve largely independently from the primary tumour, and that they accumulate genetic alterations until they eventually grow out.
- We suggest using the term *dissemination* for the processes of leaving the primary lesion and homing to and surviving in the new environment, and the term *metastasis* for the successful growth of a cancer cell to a clinically detectable, distant colony. Thereby, dissemination is necessary but not sufficient for lethal manifestation of metastasis, and metastatic growth can occur years after successful homing to a distant site, possibly triggered by intrinsic and extrinsic factors that were not present at the time of dissemination.
- The progression of a micrometastasis to a clinically manifest metastasis depends at least partially on its ability to induce a blood supply. As a consequence of the angiogenic switch, the dormant micrometastasis downregulates inhibitors of angiogenesis and starts to express angiogenic proteins.
- There is growing evidence that cellular senescence in aging tissues is associated with a secretory phenotype of the microenvironment that may stimulate neoplastic growth of epithelial cells.

Abstract

This chapter summarizes current concepts of dissemination (the processes of leaving the primary lesion and homing to and surviving in a new environment) and metastasis (the successful growth of a cancer cell to a clinically detectable, distant colony) and the role of microenvironmental as well as systemically acting factors in these processes. We review recent research on genotypes and phenotypes of early disseminated cancer cells and discuss the role of tumour dormancy, angiogenesis, and genetic background, aging and the immune system in metastasis.

6.1 Introduction

In most cancer literature metastasis is referred to as the 'major cause of cancer mortality', while the process of metastatic spread and the mechanisms involved are rarely addressed. In fact, very few research groups have been focusing on the genetics and epigenetics of disseminating cancer cells, the role of the microenvironment for homing, survival and colonization, and the selection pressures acting on tumour cells that are leaving the primary tumour (Fig. 6.1). In contrast, metastasis was and still is often viewed as the inevitable consequence of tumours that have become just too large to persist as a local disease. During recent years, this popular opinion has been challenged by new and interesting data. It is the goal of this chapter, to introduce the emerging concepts to scientifically interested physicians, as they might stimulate innovative translational research.

6.2 Metastatic Dissemination of Tumour Cells

6.2.1 Clinical Courses and Experimental Data from Primary Tumours and Metastases Do Not Enable a Coherent Understanding of Metastatic Progression

For at least one century, the prevailing view has considered metastasis as a late and final step in cancer progression. There are indeed good intuitive reasons for this opinion, such as that most cancer patients die from metastases and not from their primary disease or that early surgery is often the only chance to cure the patient. Also, supportive experimental data have been gathered, such as somatic genetic changes accumulating during local cancer progression—many of which can also be identified in metastases. The observed accumulation of genetic aberrations during local tumour growth (Fearon and Vogelstein 1990) was consequently extrapolated to systemic progression and, repeatedly, 'metastogenes' have been proposed, such as CD44v or PRL-3, thought to switch on a metastasiogenic program of invasion, dissemination, colonization and metastatic outgrowth (Saha et al. 2001; Zoller 1995). These data were consistent with another very influential observation. During transplantation experiments it was noted that only rare variant cells within the tumour will give rise to metastases (Fidler and Kripke 1977), so that a simple comparison between primary tumours and metastasis should enable the identification of those additional hits in the genome that transform a primary tumour cell into a metastatic cell. However, a recent study could not convincingly demonstrate the existence of metastasis-specific genes despite almost complete

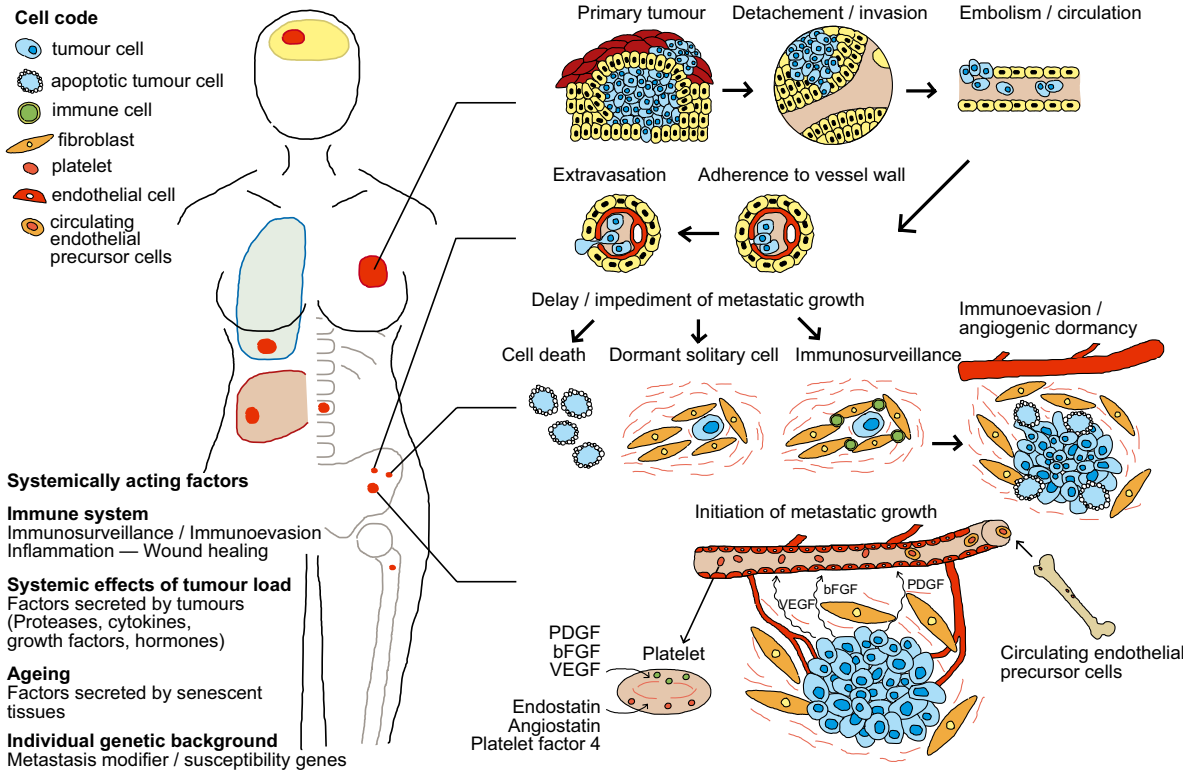

Fig. 6.1. A holistic view of metastasis. *Left side* lists systemically acting factors that have been shown to influence the growth of metastases. *Right side* depicts the individual steps of metastatic spread. Tumour growth, invasion, intravasation and extravasation precede metastatic colonization at a distant site. Tumour cells may then undergo cell death or remain dormant for many years, either by the inability to leave the G_0 cell cycle state at all, by control of immune cells or, after collapse of the immunosurveillance, by an inability to induce angiogenesis. This dormancy period may be overcome when tumour cells accumulate advantageous genetic alterations that enable colonization. Tumours resuming metastatic growth have to induce angiogenesis in order to form a detectable metastasis via the secretion of cytokines. New endothelial cells do not all originate from neighbouring vessels. A few arrive as precursor bone-marrow-derived endothelial cells. Endothelial growth factors are not all delivered to the local endothelium directly from tumour cells. Some angiogenic regulatory proteins (both pro- and antiangiogenic) are scavenged by platelets, stored in alpha granules and seem to be released within the tumour vasculature. *PDGF* platelet-derived growth factor, *bFGF* basic fibroblast growth factor, *VEGF* vascular endothelial growth factor. (Figure modified from Aguirre-Ghiso 2007; Fidler 2003; Folkman 2007)

sequencing of the genomes of metastases and primary tumours (Jones et al. 2008).

It is not only the failure of large genome screens of advanced, highly aggressive tumours undertaken in the search for the 'metastogenes' that challenge the late-metastasis concept but also this view implies some fundamental inconsistencies. For example, it is well known by clinicians that metastases also develop in patients with small cancers or even in the absence of detectable primary tumours (so-called cancer of unknown primary, which ranks among the ten most frequent cancer diagnoses; Abbruzzese et al. 1994; van de Wouw et al. 2002). Furthermore, statistical evaluation of data from the Munich Tumour Registry comprising more than 12,000 breast cancer patients indicated that the process of metastasis might have already been initiated 5–7 years before clinical diagnosis of the primary tumour (Engel et al. 2003). Perhaps even better known is the successful prediction of the clinical outcome of a patient using gene expression profiling on microarrays. As the risk of metastatic disease within the first 5 years after surgery can be predicted with high accuracy from the gene expression profile of the primary tumour (Sotiriou and Piccart 2007), it was concluded that metastatic proclivity must be represented in the gene expression profile of the dominant cell clone. Thus, the metastatic

potential cannot be acquired late in local progression in rare variant cells, but should be generated early (Bernards and Weinberg 2002).

6.2.2 Studying the Precursor Cells of Metastasis

It is relatively easy to generate lists of inconsistencies for every current model of metastasis, in particular as the concepts are now challenged by the cancer stem cell (CSC) hypothesis (Reya et al. 2001), which adds as a further complication a hierarchy of tumour cells, generated by non-genetic mechanisms, to the progression puzzle. The CSC hypothesis assumes that only rare subpopulations of tumour cells are driving the growth and spread of malignant cancers. These tumour cells are thought to be derived from organ-specific stem or progenitor cells and therefore are phenotypically and functionally defined. As the genetics of the CSCs in comparison to more differentiated and supposedly less relevant tumour cell populations have not yet been determined, concepts based on genetic data cannot yet be linked to this new paradigm. For all of these reasons, it is necessary to bridge the gap between primary tumours and metastases by analysing metastatic progenitor cells. To detect such cells from epithelial malignancies, various epithelial markers have been used in organs comprising only cells of mesenchymal origin, such as blood, bone marrow or lymph nodes. Cytokeratins are the most specific and currently also the most sensitive markers to detect single disseminated tumour cells (DTCs) in bone marrow, while the epithelial cell adhesion molecule (EpCAM; CD326) is favoured for the analysis of DTCs in lymph nodes, and for the detection of circulating tumour cells (CTC) in the blood stream several markers are in use (Pantel et al. 2008). When cell-based detection systems are used, DTCs can be isolated and analysed.

So far, mostly genetic data have been generated and current knowledge about the phenotype of DTCs is very circumstantial. There are three reasons for this:

1. Disseminated tumour cells are extremely rare. In patients without clinically manifest metastases, only 1–2 marker-positive cells are detected in bone marrow or histopathologically tumour-free lymph nodes per one million bone marrow or lymph node cells.
2. Initially, it was of the utmost importance to establish the malignant origin of the cytokeratin- or EpCAM-positive cells by genetic proof.
3. Phenotypic analysis of DTCs was restricted to double-staining approaches and therefore did not enable comprehensive assessment of expressed genes. The genomic analysis confirmed the malignant origin of EpCAM- and cytokeratin-positive cells and provided conceptually very important insights.

6.2.3 Dissemination Can Be an Early Event in Malignant Cancers

A very puzzling observation in breast cancer patients without metastasis was the finding that DTCs from bone marrow generally display lower numbers of chromosomal aberrations than the matched primary tumours (Schardt et al. 2005; Schmidt-Kittler et al. 2003). This finding was in obvious conflict with the Fearon and Vogelstein model predicting: (1) genetic changes in addition to those in the primary tumour and (2) metastases as being derived from the most advanced and dominant clone of the primary tumour. The conflict with the second prediction arose from the fact that when patients receive curative surgery and cytokeratin-positive DTCs are detected in bone marrow the patients are at high risk for relapse (Braun et al. 2005). Thus, the survival data pointed to a high relevance of DTCs and made it difficult to dismiss the genetically less advanced cells as irrelevant. The failure to identify DTCs displaying the genetic changes of the dominant clone in the primary tumour indicated that either rare cells from the primary tumour disseminate or that the DTCs are derived from earlier stages of cancer development. The latter reasoning was supported by the observation that in some cases DTCs displayed completely normal karyotypes, although chromosomal aberrations emerge already in premalignant lesions. Even in these cells genetic analyses with higher resolution proved the malignant origin and uncovered in some cases clonal aberrations shared with the primary tumours (Schardt et al. 2005). The genetic data therefore indicated that dissemination of tumour cells begins very early after transformation, a hypothesis that could recently be confirmed in mouse models and ductal carcinoma in situ (DCIS) patients (Husemann et al. 2008).

6.2.4 Genetic Heterogeneity During Minimal Residual Disease

Patients without metastasis at diagnosis will eventually die from systemic cancer in 20–95% of cases, depending on the tumour type, although the primary tumours have

been completely resected. This high rate of treatment failure has put adjuvant systemic therapies into the centre of clinical attention. However, while aiming at the prevention of lethal metastasis by early eradication of DTCs, these systemic therapies are administered generally in a blind way. In current clinical practice there is no effort to directly analyse the target cells of adjuvant therapies for selection of the therapeutic regimen. In contrast, it is assumed that the cells will somehow respond like the primary tumour cells (in drug response assays, using cultured primary tumour cells) or at least that molecular targets are identically expressed in DTCs as in primary tumours. The latter rationale underlies the *HER2* analysis of primary tumours to identify patients suitable for anti-*HER2*-based (e.g. trastuzumab) therapies (Piccart-Gebhart et al. 2005; Romond et al. 2005). However, only 50% of the patients with *HER2* amplification respond to adjuvant trastuzumab and the predictive power of primary tumour analysis is currently unclear. In fact, primary tumours as surrogate markers for therapy prediction are questionable for several reasons:

1. As stated above, primary tumours and DTCs diverge genetically, not only for the number of aberrations, but also for their specific nature. This has been shown for copy number changes (Schmidt-Kittler et al. 2003; Stoecklein et al. 2008) and point mutations (Klein et al. 2002).
2. During minimal residual disease, DTCs of an individual patient are genetically very heterogeneous, at least in breast cancer (Klein et al. 2002).
3. Disseminated tumour cells diverge not only from the primary tumour but also when taken from different organs. A genetic comparison of DTCs from lymph nodes and from bone marrow in oesophageal cancer patients revealed selection of different genetic changes depending on the organ from which the cells were isolated (Stoecklein et al. 2008).
4. The same genetic defect (e.g. *HER2* amplification) had different prognostic impact when identified in primary tumours and DTCs. In DTCs of oesophageal cancer patients, amplification of *HER2* was a strong predictor of poor outcome, while no prognostic role in the primary tumours could be established. Moreover, the presence of *HER2* amplification in the primary tumours was not associated with its presence in DTCs (Stoecklein et al. 2008). From these data it can be concluded that primary tumours may often be poor surrogate markers for the genetics of DTCs and thereby for response to adjuvant therapies.

6.2.5 Clonal Expansion of Disseminated Tumour Cells Occurs Shortly Before Manifestation of Metastasis

All these findings suggest that dissemination of tumour cells often occurs early after transformation, that the DTCs evolve largely independently from the primary tumour, and that they accumulate genetic alterations until they eventually grow out. In this context, it is interesting that metastases display similar percentages of specific copy number changes as primary tumours. Although some genetic alterations are more frequently found in metastases than in primary tumours, no copy number changes have been found so far specific for metastasis in any type of cancer. On the other hand, each type of cancer is characterized by a typical set of karyotypic abnormalities (Heim and Mitelman 1995) and consequently one would expect that metastases display similar chromosomal aberrations as primary tumours, although in paired analyses of primary lesions and metastases, genetic differences are often striking (Kuukasjarvi et al. 1997; Walch et al. 2000). Thus, to date it has not been finally clarified whether chromosomal aberrations shared between primary tumours and metastases indicate convergent evolution or true clonal descent. Interestingly, when bone marrow samples of patients with metastatic disease (e.g. breast cancer; Klein et al. 2002) or in the stage of minimal residual disease of very aggressive cancers (e.g. oesophageal cancer; Stoecklein et al. 2008) are analysed, several individually isolated DTCs display very similar chromosomal aberrations, suggesting that shortly before manifestation of metastasis clonal expansion of aggressive DTCs is taking place and eventually killing the patient.

6.3 Mechanisms of Metastasis

The findings that tumour cell dissemination is an early step in systemic cancer progression and thus often takes place years before clinical manifestation of metastases and that DTCs may need additional genetic hits for further progression indicate that dissemination and metastasis must be differentiated. We suggest using the term *dissemination* for the processes of leaving the primary lesion and homing to and surviving in the new environment, and the term *metastasis* for the successful growth of a cancer cell to a clinically detectable, distant colony. Thereby, dissemination is necessary but not sufficient for lethal manifestation of metastasis, and metastatic

growth can occur years after successful homing to a distant site, possibly triggered by intrinsic and extrinsic factors that were not present at the time of dissemination. We will therefore summarize some insights into mechanisms involved at early and late stages of metastatic progression.

6.3.1 Homing and Survival of Tumour Cells at Ectopic Sites

6.3.1.1 Paget's "Seed-and-Soil" and Ewing's "Hemodynamic" Paradigm

One of the earliest observations made by scientists, who were studying metastatic progression in the eighteenth and nineteenth centuries, was the non-random pattern of target organ involvement (Table 6.1). Different primary cancers showed a more or less organ-specific pattern of metastasis. From these early discoveries eventually two concepts emerged that are still debated today: Stephen Paget's 'seed-and-soil' hypothesis and James Ewing's 'hemodynamic' hypothesis (Fidler 2003; Ribatti et al. 2006; Weiss 2000).

In 1889, Paget published his landmark paper where he proposed the 'seed-and-soil' hypothesis (Paget 1889). Paget examined hundreds of autopsy records of women with breast cancer. His analysis revealed a non-random pattern of metastasis in visceral organs and bones. Neither random scattering throughout the body nor dispersal through the general circulation sufficiently explained the observed frequencies of metastatic growth at the various sites. He therefore proposed that certain tumour cells (which he termed the 'seed') had specific affinity for the environment of certain organs (which he termed the 'soil'). He concluded that metastases formed only when the seed finds compatible soil.

Thirty years later, James Ewing challenged Paget's 'seed-and-soil' hypothesis, and proposed that the non-random patterns of metastasis are the consequence of the anatomy of the vascular system (Ewing 1928). In his concept, cancer cells growing at a primary site will enter the draining circulatory vessels and will subsequently be arrested with much higher chance in those secondary organs that are perfused by these blood or lymph vessels. Interestingly, both Paget and Ewing addressed the alternative explanation. While Paget was critical about the 'hemodynamic' hypothesis, dismissing that 'remote organs ... are equally ready to receive and nourish any particle of the primary growth', Ewing stated that 'the predilection of metastases for particular organs may be due to special nutritional requirements dependent on the varying cell metabolism', and thereby acknowledged specific microenvironmental needs of different types of tumours (Weiss 2000).

The fact that two distinct but not mutually exclusive (see below) hypotheses were proposed based on the non-random distribution of metastases suggests that there are supporting and non-supporting findings for each hypothesis (Weiss 2000). For example, many autopsy studies concur with the observation that the number of metastases is often in proportion to the blood flow from the primary site to the secondary organ. However, one cannot neglect the cases where either more or fewer metastases are detected at a distant site than suggested by blood flow alone, indicating that determinants of the microenvironment are relevant (Weiss 1992). Certain tissues, such as brain, bone or adrenals, are served by a very small fraction of the circulatory system, but they are frequent sites of metastasis for certain cancers. Other organs, such as muscle, skin or kidneys, receive a considerable supply of blood while being only sporadically colonized by cancers (Ribatti et al. 2006). However, the strong tendency of colon cancer cells to metastasize to liver may be the consequence of the fact that cancer cells enter the portal vein, which drains the lower gastrointestinal tract and perfuses the liver. Even if circulating colon cancer cells colonize the liver with low efficiency only, the high number of cancer cells trapped in the capillary beds of the liver may ensure over time that some of them will start to grow into metastases (Weinberg 2007). Additional challenges

Table 6.1. Preferential sites of metastasis for different types of carcinoma (Nguyen and Massague 2007)

Tumour type	Preferred metastatic sites
Breast (ER+)	Bone
Breast (ER−)	Visceral organs
Lung (SCLC)	Different organs
Lung (NSCLC)	Contralateral lung, brain, liver, bones, adrenals
Prostate	Bone
Pancreas	Liver
Colon	Liver, lungs, peritoneal cavity
Ovarian	Peritoneal cavity

ER oestrogen receptor, *SCLC* small cell lung carcinoma, *NSCLC* non-small cell lung carcinoma

for the 'hemodynamic' concept of Ewing included some cases of lymphatic metastasis that needed to be explained by 'retrograde lymphatic embolism', a reversal of lymphatic flow, due to obstruction of lymphatic vessels. Likewise, circulating cancer cells are often not trapped in capillaries of the first encountered organ, but appeared elsewhere. Here, the existence of arterial–venous shunts, large-bore direct connections between two parts of the circulatory system, was used as an explanation. Finally, it was recognized that it is not easy to discriminate whether the delivery of cells into the target organ occurred through veins or arteries.

On the other hand, the 'seed-and-soil' hypothesis is in need of an adequate explanation as to why contralateral metastases in paired organs, e.g. in breast or kidney cancer, are unusually rare. One would expect that the best suited 'soil' for metastasizing breast or renal carcinoma cells is the contralateral mammary or renal tissue, respectively. Thus, to rescue the 'seed-and–soil' concept in the absence of contralateral metastases one has to postulate that the normal organ does not provide an optimal soil for cancer cells. Consequently, it has been suggested that the microenvironment of cancer cells at the primary lesion is different from that of the originating tissue and that the tumour cells that grow in this changed microenvironment develop the phenotype, which enables them to survive (Weinberg 2007). Moreover, migratory, 'metastatic' cancer cells may be unsuited to survive in the healthy environment of their tissue of orgin, in addition to not being suited to survive in the changed environment of the primary site.

While the rate of perfusion of an organ was relatively easy to assess and thereby Ewing's hypothesis perfectly testable, seed and soil factors have remained unknown for a long time. Recently, chemotactic factors secreted by target organs, molecules mediating adhesion between cancer cells and target-organ cells, and cellular interactions between cancer cells and endothelial cells in target organs were identified as critical determinants (Muller et al. 2001; Weinberg 2007). It has also been shown that endothelial cells in different tissues express tissue-specific molecules on their luminal surfaces, which may interact with binding partners at the surface of circulating tumour cells (Pasqualini and Ruoslahti 1996). Interestingly, cancer cells seem to favour inflammatory sites and it is very possible that sites of chronic inflammation within the body are hospitable sites for metastatic cells (Weinberg 2007).

The availability of large-scale gene expression profiling has enabled further molecular insights into site-specific metastasis. Repeated rounds of tumour cell injection into mice and subsequent isolation of metastases from bone and lung selected patterns of expressed genes that supported site predilection in this model. Tumour cells expressing the 'lung-colonizing' signature did not home to the bone, and vice versa (Kang et al. 2003; Minn et al. 2005). Genes upregulated in bone-colonizing cells included interleukin-11, chemokine receptor CXCR4, connective tissue-derived growth factor and matrix metalloproteinase/MMP1 (collagenase 1), while the, and lung-colonizing cells characteristically expressed epidermal-growth-factor family member epiregulin, the chemokine GRO1/CXCL1, the matrix metalloproteinases MMP1 (collagenase 1) and MMP2 (gelatinase A), the cell adhesion molecule SPARC, the interleukin-13 decoy receptor IL13Rα2 and the cell adhesion receptor VCAM1. While the signatures can also be detected in some primary tumours, it is currently unclear whether they are indeed functionally relevant during the homing or outgrowth of DTCs of breast cancer patients. It will be interesting to see whether DTCs from bone marrow express the identified bone signature genes.

In summary, both Paget and Ewing identified fundamental principles that govern the probability of metastasis at a distant site, which depends on the frequency with which circulating cancer cells are mechanically arrested in an organ as well as the ease with which they are able to colonize it (Weinberg 2007).

6.3.2 Tumour Dormancy

A major difference between mouse models of metastasis and the clinical course of patients is the speed at which metastasis manifests. In xenotransplantation experiments, only a few days to weeks span the time between tumour cell injection and metastasis; in transgenic animals this period may be extended to months. In patients, tumour cell dissemination may often occur early after transformation of the primary lesion and therefore the time from homing to a distant site to manifestation of metastasis may, in most cases, be measured in years. Traditionally, a latency period after curative removal of the primary growth until clinical detection of metastasis that lasts longer than 5–6 years is termed tumour dormancy (Hadfield 1954; Willis 1952). Therefore, even if the metastasis-founder cell disseminates the day before surgical removal of the primary tumour, it is clear that human tumour cells usually do not initiate metastatic growth immediately after arrival but rest there for various periods of time. Our knowledge of the mechanisms regulating this dark stage of cancer progression is currently very limited but a better understanding may pave ways for innovative therapeutic approaches.

Until recently, the fact that single DTCs and micrometastases are difficult to detect and are extremely rare has hampered the study of dormancy. Therefore, current thinking is derived from experimental models and extrapolation of clinical observations. Once disseminating tumour cells arrive at the distant site they may experience one of three fates: they may die, they may remain viable but quiescent or they may proliferate to form micrometastases (Fig. 6.1). The progression of a micrometastasis to a clinically manifest metastasis depends at least partially on its ability to induce a blood supply. So, the net result of dormancy on a clinical level derives from the inability to start proliferation at a rate that exceeds apoptosis or from the failure to induce angiogenesis (Holmgren et al. 1995). Immune reactions have been proposed to control the outgrowth of DTCs. However, experimental evidence for tumour surveillance regulating the latency period of systemic cancer is sparse (Aguirre-Ghiso 2007), although it was shown that patients with DTCs in their bone marrow had more memory CD4 T cells and more CD56(+) CD8 T cells than patients with tumour cell-negative bone marrow (Feuerer et al. 2001).

One explanation for prolonged latency periods after homing to a distant site may be provided by the genetics of DTCs. As mentioned before, tumour cells do not disseminate in a state of full malignancy but have to acquire additional genetic hits (Klein and Holzel 2006). The time needed to acquire such genetic hits may be relatively long as the majority of DTCs does not seem to be in the cell cycle (Pantel et al. 1993). Microenvironmental factors are likely to influence the progression of DTCs. Upon lodging in a non-orthotopic distant site DTCs must interpret the new environment, but very little is known about these first cellular interactions and it can only be speculated whether DTCs home to specific niches. However, there is first experimental evidence that primary tumours secrete factors [such as vascular endothelial growth factor (VEGF) or placental growth factor (PlGF)] that mobilize hematopoietic progenitor cells to various metastatic sites. These hematopoietic progenitor cells express VEGF receptor 1, preferentially localize to areas of increased fibronectin (synthesized by resident fibroblasts) and alter the local microenvironment, which leads to the activation of integrins and chemokines, such as SDF-1, which eventually promote attachment, survival and growth of circulating tumour cells (Kaplan et al. 2005). Such premetastatic niches are thought to promote tumour progression. However, it has also been suggested that the microenvironment forces DTCs into a more differentiated state (Aguirre-Ghiso 2007) and thereby induces dormancy.

6.3.3 Tumour Growth at Ectopic Sites

6.3.3.1 The Need for Angiogenesis

Angiogenesis is a prerequisite for the progression of a metastatic colony to a manifest metastasis. As the metastasis exceeds a certain size, the supply of nutrients and oxygen is hampered, and the end products of metabolism cannot diffuse out of colony easily. It is well-accepted that a primary tumour or metastasis can grow to a size of approximately 1 mm^3 and obtain sufficient supply of oxygen and nutrients by diffusion. Tumour growth beyond this size demands vascularization by means of angiogenesis (Bohle and Kalthoff 1999). The development of a vascular supply is a critical step that has been termed the 'angiogenic switch' (Hanahan and Folkman 1996). As a consequence of the angiogenic switch, the dormant micrometastasis downregulates inhibitors of angiogenesis (such as thrombospondin I) and starts to express angiogenic proteins [e.g. basic fibroblast growth factor (bFGF) and VEGF] (Naumov et al. 2006).

In order to create capillary sprouts, endothelial cells must proliferate, migrate and penetrate stroma, usually attracted by factors secreted by the growing micrometastasis (Bohle and Kalthoff 1999). In some cases, endothelial progenitor cells are recruited from bone marrow (Naumov et al. 2006) and the newly formed capillaries differ in cellular composition, permeability, stability, and regulation of growth from normal blood vessels (Bohle and Kalthoff 1999; Schulz 2005). The induction of angiogenesis is mediated by promoting and inhibiting molecules secreted by both tumour and cells from the microenvironment (Fig. 6.1). The balance of these secreted factors will determine whether angiogenesis will occur. During expansion of the tumour mass, some cells will lose oxygen supply and become hypoxic. Hypoxia will lead to an increase of hypoxia inducible factor (HIF) that will upregulate synthesis of proangiogenic proteins (Naumov et al. 2006). The switch of the tumour cell into the angiogenic phenotype leads to overexpression of angiogenic promoters that will enable recruitment of extracellular matrix and endothelial and other cells needed for angiogenesis. The most potent proangiogenic factor is VEGF, which is upregulated in the majority of human cancers and is a negative predictor of patients' prognosis (Bohle and Kalthoff 1999). Other proangiogenic factors are platelet-derived growth factor (PDGF), bFGF and nitric oxide synthase (NOS) (Bohle and Kalthoff 1999; Naumov et al. 2006). The often observed peritumoral inflammatory reaction

also promotes angiogenesis through cytokines secreted by leukocytes (Bohle and Kalthoff 1999).

Since angiogenesis is important for the transition of an indolent to a malignant systemic disease, it is an attractive target for anticancer therapy. Therefore, a number of angiogenesis inhibitors and antibodies have been developed and are either introduced in clinical practice or are undergoing clinical trials. Examples include bevacizumab, an antibody that neutralizes VEGF and was approved by the FDA for treatment of colorectal cancer, or endostatin, a broad-spectrum angiogenesis inhibitor targeting several positive and negative regulators of angiogenesis (Abdollahi et al. 2004). Currently more than 40 new drugs whose central mode of action is thought to be inhibition of angiogenesis are in clinical testing (Folkman 2007) and we will soon know how effective and how robust this therapeutic approach will be.

6.4 Systemically Acting Factors: Genetic Background, Aging and the Immune System

Perhaps the major lesson that has been learned during recent years is that metastasis is not a consequence of seeding of fully autonomous cells. There is rarely such thing as a fully malignant cell ready to start growing independently at the distant site upon arrival. The ability of a tumour to form a metastasis is influenced by many interacting factors and is not only a function of somatic events in the tumour cells, but also of the constitutional genetic differences between individuals, affecting the gene expression of tumour cells in transit and at secondary sites. Thus, our view on metastasis must become more holistic and not surprisingly there are already data showing that metastasis is influenced by systemically acting factors (Fig. 6.1).

6.4.1 Metastasis and Genetic Background

Paget proposed from early on that metastasis is the result of characteristics of the seed *and* the soil. Clearly, the genetic background of each patient comprises information on tumour cell-intrinsic and microenvironmental factors influencing the manifestation of metastasis. Therefore, it is not surprising that there is a rapidly growing literature linking germ line polymorphisms to the emergence of metastasis. In a landmark experiment, it was shown that the same aggressive oncogene (polyoma middle T-antigen under control of a mammary specific promoter) in different mouse strains results in different rates of tumour growth and metastasis-free survival of the animals (Lifsted et al. 1998). Moreover, a frequently used metastasis predictive gene signature (Ramaswamy et al. 2003) is differentially expressed between *normal* mammary tissue of mice with metastasis-prone and metastasis-reduced genetic background (Yang et al. 2005), indicating that many if not all of the prognostic gene expression signatures may reflect more the response of a genetic background to malignant transformation than the consequence of specific somatic mutations. In humans, genetic polymorphisms and haplotypes are increasingly found to be associated with a propensity to systemic progression that potentially influence various metastatic mechanisms such as invasion (Sun et al. 2006), angiogenesis and stress response (Menendez et al. 2006), and the interaction with the microenvironment (Crawford et al. 2008). Whether systematic searches for metastasis susceptibility genes will have an impact on cancer screening and preventive measures has to be awaited.

6.4.2 Metastasis, Immune System and Ageing

Individual differences in the ability of the immune system to protect against or promote cancerous transformation or metastasis could likewise be a genetic trait. Currently, little is known about the role of the immune system in protecting specifically against systemic cancer spread (see above). However, for colorectal cancer, it was recently shown that the immune reaction at the tumour site determined clinical outcome regardless of the local extent and spread of the tumour. A weak adaptive immune reaction correlated with a very poor prognosis even in patients with minimal tumour invasion. Conversely, a high density of adaptive immune cells correlated with a highly favourable prognosis whatever the local extent of the tumour and the invasion of regional lymph nodes (Galon et al. 2006). As the study included a high number of patients representing a large fraction of the genetic heterogeneity of colorectal cancers, it is unlikely that the protective action of the immune system was limited to a subset of patients with specific oncogenic mutations. Rather, as in colorectal cancer no molecular marker has ever been shown to outperform the TNM staging system in a similar way, the data apparently demonstrate the amazing capability of the individual immune response of some patients to keep genetically instable tumours in check. Despite the phenomenon of immunoediting, i.e. selection of tu-

mour cells with reduced immunogenicity (Dunn et al. 2002), the beneficial effect of the adaptive immunity appeared to persist throughout tumour progression from stage I disease to stage III disease. The data provide an interesting example of the coevolution of cancer and protective defence mechanisms, both being strongly determined by the individual's genetic constitution.

Finally, while this chapter cannot address external influences, such as carcinogens, cancer-promoting agents, infections, irradiation and systemic cytotoxic therapies, on carcinogenesis and specifically on metastasis, it should not end without mentioning one additional systemically acting factor that is likely to be the subject of scientific scrutiny in the coming years: ageing. While cancer incidence increases with age, it has also been noted that growth rates of breast cancers are often slower in older patients. This seems to be associated with a significant reduction of axillary lymph node metastases, vascular invasion and lymphoplasmacytic stromal reaction with increasing age (Fisher et al. 1997). In a comparison of metastatic efficiency of B16 melanoma cells injected into young, old and parabiotic (i.e. surgically unified old and young) mice, it was possible to directly measure the effect of age on the outgrowth of lung metastases. In unpaired mice, the number of metastatic colonies in the lungs was ten times higher in young than in old mice. However, in parabiotic mice, the number of metastases in young mice was almost comparable with that of unpaired young mice, while the number of metastases in old mice approached the level of young mice. Although the number remained stable in young parabiotic mice, their size was reduced. In old parabiotic mice, almost exclusively small colonies were observed as in unpaired old mice. The authors concluded that in these experiments the implantation of early metastatic colonies in the lung depends on systemic humoral factors while their growth is mainly dependent on local factors in the microenvironment and that both effects are modulated by age (Hirayama et al. 1993). As there is growing evidence that cellular senescence in aging tissues is associated with a secretory phenotype of the microenvironment that may stimulate neoplastic growth of epithelial cells (Campisi 2005), upcoming studies have to unravel the molecular changes of the aging host and their influence on the manifestation of metastatic disease in patients.

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