Cancer Dormancy: A Model of Early Dissemination and Late Cancer Recurrence

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Abstract
Cancer dormancy is a stage in tumor progression in which residual disease remains occult and asymptomatic for a prolonged period of time. Dormant tumor cells can be present as one of the earliest stages in tumor development, as well as a stage in micrometastases, and/or minimal residual disease left after an apparently successful treatment of the primary tumor. The general mechanisms that regulate the transition of disseminated tumor cells that have lain dormant into a proliferative state remain largely unknown. However, regulation of the growth from dormant tumor cells may be explained in part through the interaction of the tumor cell with its microenvironment, limitations in the blood supply, or an active immune system. An understanding of the regulatory machinery of these processes is essential for identifying early cancer biomarkers and could provide a rationale for the development of novel agents to target dormant tumor cells. This review focuses on the different signaling models responsible for early cancer dissemination and tumor recurrence that are involved in dormancy pathways.

Introduction
The major cause of cancer-related deaths is metastatic growth of disseminated tumor cells (DTC) from the primary tumor. Metastatic disease may occur years or even decades after successful treatment of the primary tumor by surgery and adjuvant treatment (1, 2). It has been proposed that this latency period is due to a clinical phenomenon called tumor dormancy. Dormant tumor cells may exist in a quiescent state for many years as solitary tumor cells [i.e., cellular dormancy (3–5)], or as micrometastases whose cellular proliferation is counterbalanced by apoptosis [i.e., tumor mass dormancy (6, 7)]. Consequently, tumor dormancy is a stage in cancer progression in which residual disease is present but is not clinically apparent.

During carcinogenesis, tumor cells accumulate genetic alterations that lead to immortalization (loss of function of TP53, retinoblastoma 1 (Rb), p16, and/or gain of telomerase) and transformation (gain of RAS or BRAF mutations, and ERBB2 amplification). Recent evidence indicates that DTCs have different and fewer genetic alterations compared with primary tumor cells, suggesting the early dissemination of human cancers (8, 9). The general mechanisms that regulate the transition of DTCs that have lain dormant into a proliferative state remain largely unknown. The presence of particular genetic abnormalities acquired by dormant cells may explain the early dissemination of tumor cells, the latency state, and resistance to the conventional therapeutics used in the treatment of cancer that target actively dividing cells. However, additional mechanisms, such as the interaction of the tumor cell with its microenvironment, limitations in the blood supply, or an active immune system, can also explain the regulation of the growth of dormant cells.

By the time of diagnosis, DTCs can be found in secondary organs such as bone marrow and lymph nodes (10). The detection of circulating tumor cells may explain the dissemination from primary tumors to target organs (11). The ability of these cells to become tumor metastases is a complex mechanism that may be explained by the tumor dormancy process (Fig. 1). The mechanisms that are involved in tumor dormancy, in relation to tumor recurrence and sensitivity to therapeutic interventions, represent an area of major interest and investigation. This review discusses the importance of cancer dormancy and how this model is part of disease progression and therapeutic response.

Influence of the microenvironment on cancer cell dormancy
Recent evidence indicates that the tumor microenvironment is a critical regulator of cancer progression (12–15) and a major factor in determining the survival and growth of DTCs at preferential metastatic sites (16). Several of the
important factors or pathways of the microenvironment that influence tumor cell dormancy are discussed below.

Dormant tumor cells are in immediate contact with the extracellular matrix (ECM) through integrin signaling, which regulates aspects of tumor cell growth, migration, differentiation, and survival (17, 18). Integrins are heterodimeric proteins made up of α- and β-subunits. At least 18 α- and 8 β-subunits have been described in mammals. Integrin family members are membrane receptors that are involved in cell adhesion and recognition in a variety of processes, including the metastatic diffusion of tumor cells.

The metastasis-associated urokinase receptor (uPAR) causes tumor growth by interacting and activating the fibronectin receptor α5β1-integrin (19). This complex in turn recruits focal adhesion kinase (FAK) and EGFR, which promotes adhesion to fibronectin and propagates mitogenic signals through the Ras extracellular signal-regulated kinase (ERK) pathway, leading to proliferation (Fig. 2). An in vitro study showed that the downregulation of uPAR and loss of integrin function reduced proliferative signals from the fibronectin-rich microenvironment, causing a shift from the state of tumorigenicity to dormancy in human carcinoma cells (20, 21). Furthermore, blocking of uPAR, β1-integrins, FAK, or EGFR, alone or in combination, resulted in in vivo tumor suppression, which was shown to be due to induction of tumor cell dormancy (22, 23).

ERK1/2 signaling in vivo revealed that dormancy resulted from an almost complete inhibition of the Raf– MEK–ERK pathway and induction of a G0–G1 cell-cycle arrest, as is present in quiescent cells (24). The p38/c-Jun N-terminal kinase (JNK) mitogen-activated protein kinase (MAPK) signaling cascade was shown to be involved in this...
growth arrest by acting as a tumor suppressor through the attenuation of several oncogenic signals and regulation of different tumor suppressor pathways [e.g., TP53- and Rb-dependent (25)]. The disruption of the uPAR complex activates the p38 MAPK signaling pathway. Proliferation in primary and secondary tumors requires a high ERK1/2 to p38 MAPK signaling ratio, whereas the opposite favors cellular dormancy (26). Thus, the proposed molecular mechanisms of the growth inhibition that occurs in dormancy involve either activation of the p38 MAPK pathways or inhibition of the ERK1/2 MAPK pathways.

The ECM structure is dynamic and can be degraded by the family of enzymes known as matrix metalloproteases (MMP). These enzymes are mainly secreted by stromal cells (27) or by heparanase, an endoglycosidase enzyme that cleaves heparin sulfate chains, which are preferentially expressed and secreted by tumor cells (28). Thus, through the action of MMPs, the microenvironment may contribute to tumor dormancy or its switch to metastatic growth. The secretion and expression of MMPs by leukocytes and macrophages can lead to the release of angiostatic factors from the ECM that can inhibit angiogenesis and metastatic growth. These antiangiogenesis factors include endostatin, restin, arrestin, the 3 chains of collagen IV, and macrophage elastase (MMP-12; refs. 29–32). In the same way, stromal MMPs may release cytokines and angiogenic factors that are sequestered to ECM molecules, such as fibroblast growth factor (FGF) and VEGF and initiate the angiogenic switch needed to transition from micrometastatic dormancy to metastatic growth (33). MMPs may also contribute to the formation of a permissive niche for the transition from dormancy to metastatic growth. For example, changes in
Metastasis process and cancer dormancy

Metastasis is a complex, multistage process in which malignant tumor cells spread from the primary tumor to secondary organs. Tumor cells acquire an invasive phenotype to invade the stromal tissue and disrupt the vascular endothelium (intravasation). Once in the blood, the DTCs must survive in the circulating environment and escape physical damage and attack by the immune system. After the tumor cells arrest or adhere to vessel walls, they invade through the capillary wall (extravasation). Finally, DTCs must adapt to the new microenvironment of the secondary site and start to form micrometastasis or reprogram into a quiescent state, which can last for years. There is growing evidence that several metastasis suppressor genes that are involved in the induction of tumor cell growth arrest, decreasing the formation of distant metastasis (47).

The proteins of metastasis suppressor genes are defined by their ability to prevent the development of metastasis by inducing apoptosis or dormancy once the cells have lodged at the secondary site. The proteins encoded by these genes participate in a diverse range of signaling pathways, and in some cases they inhibit not just one but multiple steps in the metastatic cascade (40).

KISS-1 metastasis-suppressor (KISS1) encodes for a secreted propeptide that is processed into kisspeptins that possess neuroendocrine effects (41). Kisspeptins bind to G protein-coupled receptor 54 (GPR54) and regulate events downstream of cell-matrix adhesion (42), perhaps involving cytoskeletal reorganization to block metastases through the induction of dormancy of solitary cells. In an in vivo study, cutaneous melanoma cells were injected into athymic nude mice (43). Cells that expressed kisspeptins remained dormant in multiple organs and were transfected without any detectable growth after injection. However, cells that were transfected with a deletion variant of kisspeptin formed detectable macrometastases in the lung, bone, kidney, and eye. Although the expression of KISS1 is transcriptionally regulated by CRSP3 (44), the mechanism through which KISS1 induces dormancy does not appear to be dependent on its receptor, GPR54.

Kangai 1 (Kai1/CD82) was first described as tetraspanin, a cell-surface transmembrane protein that forms complexes with integrins and participates in the inhibition of cancer cell migration and invasion (45, 46). Further studies suggested that Kai1 binds to Duffy antigen chemokine receptor on the surface of vascular endothelial cells that may be involved in the induction of tumor cell growth arrest, decreasing the formation of distant metastasis (47).

In different cancer models, including melanoma, colon, breast, and lung cell carcinomas, investigators have shown suppression of metastasis through the Nm23–1H

Table 1. Putative metastasis suppressor genes involved in the dormancy process

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function related to dormancy</th>
<th>Dormancy process</th>
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<tr>
<td>Kiss1</td>
<td>Regulates events downstream of cell-matrix adhesion, perhaps involving cytoskeletal reorganization</td>
<td>Cellular dormancy</td>
<td>41, 42</td>
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<tr>
<td>CRSP3 (MED23)</td>
<td>Transcriptionally regulates Kiss1 expression</td>
<td>Cellular dormancy</td>
<td>44</td>
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<td>Kai-1 (CD82)</td>
<td>Inhibits adhesion signaling</td>
<td>Angiogenic dormancy</td>
<td>45–47</td>
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<td>NME1 (NM23)</td>
<td>Inhibits Ras signaling</td>
<td>Cellular dormancy</td>
<td>48–51</td>
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<td>MKK4</td>
<td>Activates MAPKs p38 and JNK</td>
<td>Cellular dormancy</td>
<td>53, 54</td>
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<tr>
<td>MKK6</td>
<td>Activates MAPK p38</td>
<td>Cellular dormancy</td>
<td>54</td>
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<tr>
<td>MKK7</td>
<td>Activates MAPK JNK</td>
<td>Cellular dormancy</td>
<td>53</td>
</tr>
<tr>
<td>BRMS1</td>
<td>Reduces the metastatic potential but not the tumorigenicity.</td>
<td>Angiogenic dormancy</td>
<td>55</td>
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<td>SMAD7</td>
<td>Interacts with TGF-β receptor type 1</td>
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<td>SSeCKS</td>
<td>Encodes a cell-growth-related protein</td>
<td>Cellular dormancy</td>
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<tr>
<td>RhoGD12</td>
<td>Involved in cell signaling, proliferation, cytoskeletal organization, and secretion</td>
<td>Angiogenic dormancy</td>
<td>58</td>
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<tr>
<td>CTGF</td>
<td>Regulates cell adhesion, proliferation, and differentiation</td>
<td>Cellular dormancy</td>
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Abbreviations: BRMS1, breast cancer metastasis suppressor 1; CTGF, connective tissue growth factor; MED23, mediator complex subunit 23; RhoGD12, guanine nucleotide binding protein; SMAD, SMAD family member 7; SSeCKS, src-suppressed c-kinase substrate.
(NME1) protein (48–51). Although the molecular mechanism of Nm23–1H–mediated dormancy has not been fully elucidated, it is believed that Nm23–1H suppresses multiple aspects of metastasis, including invasion, tumor cell survival, and metastatic colonization. In an in vivo model, Nm23–1H appeared to interact with EDC2 lysophosphatidic acid receptor 1, a strong activator of ERK1/2, to reduce MAPK ERK1/2 activation and balance the p38:ERK1/2 signaling ratio, thus favoring dormancy (52).

Mitogen-activated protein kinase kinase 4 (MKK4) is a specific kinase that plays a role in dormancy in a micro-metastatic stage, with a dual mechanism of action through upstream activation of the MAPKs p38 and JNK. In contrast, MKK7 only activates the JNK stress pathway and MKK6 only activates p38 as a mechanism of metastatic suppression (Fig. 2). In a prostate cancer model, although micrometastatic foci were present in the lungs of mice bearing tumors that ectopically expressed MKK4 and MK7, they did not progress to gross macroscopic metastases as they did in control mice (53). A link between MKK4 and MKK7 was elucidated in subsequent experiments through the JNK pathway activation, but only in the metastatic site and not in the primary tumor, suggesting a tissue-specific role for these kinases. In an ovarian cancer model, MKK4 caused cell-cycle arrest through the activation of p38, also activated by MKK6, which induced p21 upregulation (54). These data suggest that MKK4 plays an important role in cancer dormancy at a micrometastatic stage, with a mechanism of action that may be tissue- or cell-line–dependent.

Other possible metastatic suppressor genes include BRMS1, SMAD7, SSsC5, RhoGD12, and CTGF, which were shown to selectively reduce the size of observable metastatic lesions and thus have a potential role in dormancy (55–59). Although the genes and pathways outlined above may influence dormancy by different mechanisms, the data support the conclusion that they each have an overall influence on regulation of the p38:ERK1/2 signaling ratio, which under a more activated p38 cell state promotes the quiescence of aggressive tumor cells, thereby inducing cancer cell dormancy.

**Angiogenic dormancy**

Tumor mass growth requires the recruitment of oxygen and nutrients through the formation of new functional blood vessels. The inability of tumors to induce angiogenesis in which a high proliferation rate is counterbalanced by elevated apoptosis results in a cell dormancy state referred to as angiogenic dormancy (60). In this situation, prevascular tumor cell expansion is blocked as a result of the balance between proangiogenic factors (e.g., VEGF, platelet-derived growth factor, FGF, and angiotropin) and antiangiogenic factors (e.g., endostatin, vascuostatin, and angiostatin). The transition from a predominantly anti-angiogenic factor environment and dormant cell state to a predominantly proangiogenic factor environment and progressive outgrowth of the tumor is known as the angiogenic switch (61–63).

**VEGF-A and thrombospondin-1.** Genetic alterations in the pathways that maintain angiogenic dormancy or an eventual exogenous proangiogenic signal may restore tumor growth. For example, oncogenic RAS has been implicated in sustaining tumor angiogenesis by inducing VEGF and repressing thrombospondin. In contrast, loss of function of the tumor suppressor p53 and the stress-activated kinase p38 can induce thrombospondin or repress VEGF. In several studies (7, 64, 65), investigators developed different models to show the importance of angiogenesis in the escape from dormancy and induction of tumor growth and progression. In these studies, long-term dormancy was induced by the presence of a potent inhibitor of angiogenesis (7, 64). Moreover, the addition of angiogenic factors resulted in tumor cells escaping from tumor dormancy and switching to a rapid growth state (65). The genetic characteristics of dormant and rapidly proliferating tumor cells have been also compared. Tumor cells with similar proliferation rates in vitro presented different growing phenotypes in vivo, and these differences were shown to be related to the intratumoral microvasculature patterns (66, 67). It is commonly accepted that hypoxia signaling through hypoxia-inducible factors, whose activity is regulated by oxygen-sensing prolyl hydrolases, influences the first steps of the metastatic cascade (68–70). However, emerging evidence indicates that in certain experimental situations, inhibitors of VEGF and its receptors can also promote tumor cell invasion and metastasis (71, 72). Although such effects remain controversial, genetic studies revealed that vascular-targeting strategies that avoid tumor hypoxia or even promote tumor oxygenation may prevent an invasive metastatic switch (73). Some preclinical data even showed a divergent effect between primary tumors and metastasis after the VEGF blockade (74, 75). It remains to be determined whether tumor cells migrate to a higher oxygenated environment or hypoxia signaling induces promotion of metastasis by these inhibitors.

A better understanding of the angiogenesis switch will aid in the design of therapies to either induce or maintain tumor dormancy, or, conversely, to induce cell death in residual dormant cells.

**Immunosurveillance and tumor dormancy**

During the last several decades, studies have shown a critical role for the immune system in preventing cancer initiation (76). However, its role in cancer progression remains unclear. Immunosurveillance is an additional mechanism whereby an equilibrium between the host’s immune response and dormant tumor cells could be established. The limited success of current cancer immunotherapy may be largely attributed to the poorly understood complex relationships between cancer cells and the immune system.

In addition to the immune system’s capacity to destroy cancer, it can also control cancer for long periods of time, as shown in a mouse model (77). Tumor cells in equilibrium in immunocompetent mice spontaneously escaped immune control and grew into clinically apparent tumors in immunodeficient mice. Other studies have shown a role
Role of CSCs in tumor dormancy. The tissue microenvironment and the heterogeneous nature of solid tumors dictate the survival of cancer cells and CSCs in both the primary tumor and distant metastases. Once the CSCs migrate to their site of metastasis, they may remain dormant for an extended period of time, embedded in a niche that protects them. They can be quiescent, efficiently repair any damage done to the DNA, be resistant to chemotherapy, and remain in the hypoxic environment. However, the CSCs may be activated when the microenvironment changes, and may signal for the cells to proliferate and differentiate into a tumor mass.

Clinical implications and future directions

The term “tumor dormancy” has been used for many years to describe the stage in tumor progression in which cancer cells remain occult after treatment of the primary tumor, pending subsequent growth and clinical recurrence. For example, in patients with breast and prostate tumors, recurrence is common and relapse can occur years or even decades after the initial diagnosis. It is believed that dormant tumor cells are resistant to initial therapies used with curative intent, and are responsible for recurrence and even the death of patients (80). Mathematical models suggest a period of tumor quiescence followed by a rapid growth after emergence from dormancy, rather than a slow, constant period of tumor quiescence followed by a rapid growth after death of patients (80). Increasing evidence suggests the existence of a heterogeneous subpopulation of tumor cells within tumors, commonly known as cancer stem cells (CSC; Fig. 3). CSCs may be responsible for the continued growth, invasion, and metastasis of tumors, and resistance to various commonly used chemotherapeutic treatments. Stem-cell markers represent an area of ongoing research and controversy. To date, there is no consensus as to which markers identify stem cells in different solid tumors. The functions of many of these markers are unknown, but they may play an important role in tumor cell dormancy (83). In a recent work conducted by our group, we found that common germline variants in CSC genes, including the Wnt-target genes, predicted early tumor recurrence in patients with stage III and high-risk stage II colon cancer (84). Recent research has related CSCs to a regulatory cellular process known as the epithelial–mesenchymal transition, during which epithelial cells acquire the ability to invade, resist apoptosis, and disseminate. The epithelial–mesenchymal transition may not only contribute to the self-renewal ability and drug resistance of these cells, but may also be responsible for creating and maintaining CSCs (85). Moreover, we have to consider the interaction of these tumor cells with their microenvironment. The integration of integrin intra- and extracellular signals with those originating from the growth factor receptors results in specific cellular behaviors in different biologic situations. For example, we recently identified integrin gene variants (ITGB3, ITGB1, ITGA4, and ITGA3) that predict stage-specific tumor recurrence in patients with colon cancer (unpublished data). These results may aid in the selection of subgroups of patients who might benefit from more-aggressive treatment strategies or integrin-targeted treatments. Current agents that target the αvβ3 integrins or the α4 subunit may be useful in selected patients with colon cancer and should be considered in future clinical trials.
However, much remains to be learned about whether current therapies target CSCs and dormant cancer cells, and whether new therapies can be developed to target these cells. Furthermore, the benefits versus risk to the patients, as well as the cost to the health care system, of putative chronic treatment strategies need to be carefully evaluated.

Currently, the decision to use adjuvant treatment for patients at risk for recurrence after an intentional curative surgery is essentially based on tumor characteristics. The complex cross-talk among dormant cancer cells, the microenvironment, and surrounding cells shows that cancer research is more than the study of genetic tumor changes alone. In fact, most tumors arise in tissues with injuries or chronic inflammation, which favors a malignant transformation. Thus, it is imperative to define new tumor and host pharmacogenetic signatures to better assess each patient’s risk of recurrence.

Treatment of early-stage tumors could have a greater impact on improving patient outcomes compared with treatment of systemic disease of large and vascularized tumors. However, results from recent trials (AVANT and NSABP-08) failed to show a clear benefit from the antiangiogenic therapy bevacizumab when it was given as 1 year of adjuvant treatment in early-stage colon cancer (86, 87). It is our aim to elucidate which specific patients might benefit from treatment, and the precise timing of antiangiogenic therapy that is needed to show benefit.

A potential immune-modulatory approach would be to use cancer vaccines whose immune response could inhibit tumor cells from emerging from their dormant state; however, at present, this process remains poorly understood (88). Other immunologic mechanisms, such as the induction of apoptosis in cancer cells through the activation of natural killer cells, are being studied in both hematologic and solid tumors (89).

In conclusion, a better understanding of the molecular biology of the dormancy phenomenon, including both tumor and host genetic alterations, would permit the development of long-term treatment strategies to prevent cancer progression, as well as strategies to target dormant cells.

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No potential conflicts of interest were disclosed.

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