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Perspective

Tumor Cell Dissemination: Emerging Biological Insights from Animal Models and Cancer Patients

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Circulating tumor cells (CTCs) and disseminated tumor cells (DTCs) are increasingly recognized for their potential utility in disease monitoring and therapeutic targeting. The clinical application of CTC/DTC requires better understanding of the biological mechanisms behind tumor dissemination, the survival of DTCs, and their activation to aggressive growth from dormancy. Recent research using animal models of DTCs and CTCs have provided novel insights into these processes. Here, we discuss these findings in the context of results obtained from the clinical analyses of CTCs and DTCs, which demonstrate that the animal models mimic, in many aspects, the complex situation in patients.

Many cancer patients are diagnosed with early-stage cancer with no clinical symptoms of metastasis but subsequently succumb to metastatic relapse. Circulating tumor cells (CTCs) in the blood and disseminated tumor cells (DTCs) that have already reached a secondary organ but have not yet grown to become clinical overt metastasis are frequently detected in these patients and have been linked to poor prognosis (Pantel and Brakenhoff, 2004). To exploit the window of opportunity for therapeutic intervention between initial dissemination and eventual metastatic recurrence, a better understanding of the biology and clinical relevance of tumor dissemination is needed. In this review, we will discuss novel insights into various aspects of tumor dissemination as the result of convergence in experimental animal model research and clinical analysis. These emerging themes will guide future research on DTCs and CTCs in preclinical and clinical settings, which may lead to effective therapeutic strategies to prevent metastatic relapse.

The Temporal-Spatial Dynamics of Tumor Cell Dissemination

In contrast to the traditional notion that metastasis is a late event in tumor progression, increasing evidence suggests that tumor cells can disseminate from the earliest preneoplastic lesions, sometimes even before the formation of overt primary tumors (Figure 1; Table 1). In genetically modified mouse models of pancreatic cancer, preneoplastic tumor cells were found to undergo epithelial-mesenchymal transition (EMT) and seed distant organs before frank malignancy could be detected (Rhim et al., 2012). Interestingly, induction of pancreatitis and other inflammation-associated events increased the number of circulating pancreatic cells. This observation in pancreatic cancer is consistent with an earlier report using inducible expression of oncogenes in mammary epithelial cells. Untransformed mammary epithelial cells can survive as disseminated cells in the lung and assume malignant growth upon oncogene activation (Podsypanina et al., 2008). Consistent with animal model studies, patients with benign inflammatory bowel diseases show already circulating epithelial cells in their peripheral blood (Pantel et al., 2012). Analysis of human epidermal growth factor receptor 2 (HER-2) and PyMT transgenic mice and ductal carcinoma in situ (DCIS) in breast cancer patients also showed early dissemination of tumor cells and micrometastasis (Hüsemann et al., 2008). Although it is unclear yet whether DCIS patients with DTCs have an increased risk of metastatic relapse, these findings clearly show that tumor cells in these early-stage lesions already have the capacity to survive in the blood circulation and home to distant organs. Taken together, these studies suggest that dissemination of epithelial cells can occur very early, even in premalignant lesions. It is even possible that progression of these cells may contribute to the development of cancer of unknown primary, which occurs in 0.5% and 7% of all cancer patients (van de Wouw et al., 2002). Long-term prospective follow-up studies are required to determine the fate of these disseminated cells in humans, possibly with the aid of next generation sequencing to determine the lineage relationship between disseminated cells and eventual malignancies.

The emerging concept of tumor self-seeding has also extended our understanding of the pathological impact of tumor dissemination. In addition to seeding distant metastasis, DTCs are found to have enhanced malignant features and often return to the primary tumors to accelerate their expansion (Kim et al., 2009). It might be difficult to prove that such self-seeding demonstrated in elegant mouse models also occurs in cancer patients. However, the observation that the presence of DTCs in bone marrow is correlated to the subsequent occurrence of local relapse in breast cancer patients (Braun et al., 2005; Janni et al., 2011) suggests that tumor cells may recirculate from a distant site (i.e., bone marrow) back to the primary site to give rise to recurrent tumors. Future studies comparing the genomes of DTCs at primary diagnosis with those of tumor cells in local relapse sites might reveal important clues to this open question.

Epithelial-Mesenchymal Plasticity in Tumor Dissemination

EMT has been considered as a key event for the epithelial tumor cells to lose polarity and cell-cell adhesions to initiate tumor

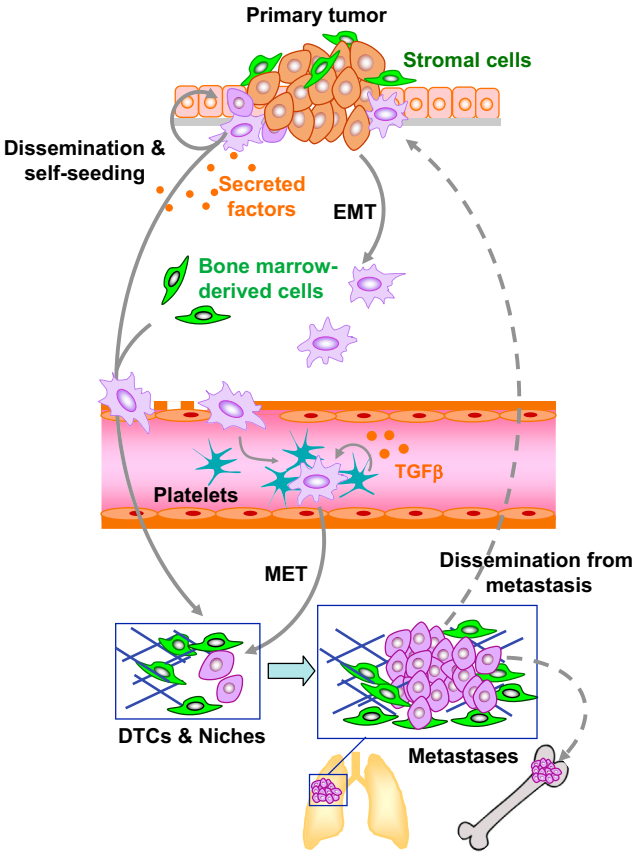

Figure 1. Schematic Representation of the Steps in the Dissemination, Survival, and Expansion of Metastatic Tumor Cells
Under the influence of stromal cells in the primary tumor microenvironment, tumor cells undergo EMT or use other means of invasion to escape from the primary tumor (Friedl and Alexander, 2011). Platelets protect CTCs in circulation and further stimulate EMT by TGF-β and NF-κB signaling. Tumor-derived factors and exosomes mobilize bone marrow-derived cells to form premetastatic niche to promote the seeding and expansion of metastasis. Stromal component at the metastasis niches enhances tumor survival, stemness, and immune evasion. CTCs derived from the primary tumor or metastasis may engage in multidirectional seeding to the primary tumor or other metastasis sites. Examples of genes mediating different steps of tumor dissemination are listed in Table 1.

dissemination (Nieto, 2011; Thiery et al., 2009). Recent discoveries of the link between EMT and stem cell properties in both normal and cancerous cells provided an appealing model to link two important enabling characteristics of tumor dissemination (Mani et al., 2008), although the separation of EMT and cancer stem cell properties has also been observed in some experimental models (Celià-Terrassa et al., 2012; Ocaña et al., 2012).

In carcinomas, EMT-related pathways are under strong influence by the surrounding microenvironment (López-Novoa and Nieto, 2009). An example of such an interaction is induction of EMT in tumor cells driven by cancer associated-fibroblasts or myeloid-derived suppressor cells, a heterogeneous population of early myeloid progenitors, immature granulocytes, macrophages, and dendritic cells with the capacity to suppress antitumor T cell responses (Giannoni et al., 2011; Toh et al., 2011), whereas pericytes exert the opposite influence (Cooke et al., 2012). In general, inflammation appears to be a strong inducer of EMT, and recent experimental studies indicated that inflammatory stroma might be even obligatory to activate EMT and dissemination in pancreatic lesions (Rhim et al., 2012).

In addition to the well-established role in protecting CTCs from mechanical and immune assaults during circulation, platelets have recently been shown to induce EMT in CTCs via transforming growth factor β (TGF-β) and nuclear factor κB (NF-κB) signaling and prime tumor cells for subsequent metastasis (Labelle et al., 2011). Moreover, platelets can also function as adaptor cells, linking tumor cells to "nursing" $CD11b^+$ macrophages, which results in the establishment of microclots that protect CTCs in blood (Gil-Bernabé et al., 2012). These experimental studies provide a possible explanation for the reduction of metastatic relapses in breast cancer patients receiving aspirin, which blocks platelet aggregation (Algra and Rothwell, 2012). Small clusters consisting of tumor cells and non-tumor cells have also been detected in the peripheral blood of cancer patients and are associated with an unfavorable outcome (Hou et al., 2012), although the cellular composition of these clusters is heterogeneous and still a subject of ongoing investigations.

The relevance of EMT in cancer patients has been a topic of heated debate (Ledford, 2011). Expression of master regulators of EMT, such as Twist1, Zeb1, Zeb2, SNAIL1, and SNAIL2/Slug in primary tumors has been linked to increased risk of metastatic relapse (De Craene and Berx, 2013). Moreover, several reports on CTCs clearly indicated that EMT occurs on CTCs in breast and prostate cancer patients and is linked to an unfavorable outcome (Bednarz et al., 2010; Joosse et al., 2012). As a consequence, many research groups are currently focusing on the development of new assays and markers that are able to detect CTCs with an EMT phenotype (Alix-Panabières et al., 2012). EMT-related markers are frequently coexpressed with different epithelial markers (Bednarz-Knoll et al., 2012; Yu et al., 2013), which implies that a considerable pool of CTCs might indeed exhibit a semimesenchymal phenotype. EMT-related markers are also found in cytokeratin-negative cells (Bednarz-Knoll et al., 2012; Gradilone et al., 2011) that lack expression of the common leukocytes antigen CD45, suggesting that these cells may represent CTCs that have undergone complete EMT. Thus, most of the current CTC technologies based only on epithelial markers might be "blind" to potentially the most dangerous cancer cells present in the circulation. Nevertheless, it is unknown how abundant might be the pools of CTCs undergoing partial or full EMT in humans, although some studies have begun to address this issue (Armstrong et al., 2011). Mesenchymal CTCs have been correlated with disease progression and resistance to chemotherapy (Mego et al., 2012; Yu et al., 2013). A caveat has to be noted for all studies that do not use confirmation of tumor cell identity by genomic markers, because most markers for (semi)-mesenchymal CTCs are not tumor-specific but can be also expressed on blood cells or circulating CD45-negative endothelial cells (Bednarz-Knoll et al., 2012).

Previously, the ability of cancer cells to undergo EMT has been linked to their stemness (Mani et al., 2008). However, recent experimental studies indicate that some tumor cell lines arrested in a mesenchymal state are more invasive but are unable to form overt metastasis (Celià-Terrassa et al., 2012; Ocaña et al., 2012; Tsai et al., 2012; Tsuji et al., 2008). Since the EMT state has been

Table 1. Representative Genes with Functional Roles in Different Steps of Tumor Dissemination

Gene(s)	Function	Reference
EMT and Invasion		
TWIST1	promotes EMT by suppressing E-cadherin expression and inducing invadopodia formation	(Eckert et al., 2011; Yang et al., 2004)
SNAIL1	promotes EMT and tumor recurrence	(Moody et al., 2005)
SNAIL2	master regulator of EMT and mammary stem cell/breast cancer stem cell	(Guo et al., 2012)
ZEB1/2	promotes EMT by repressing E-cadherin and miR-200 family expression and by increasing tumor stemness	(Spaderna et al., 2008; Wellner et al., 2009)
FOXC2	promotes EMT and tumor metastasis	(Mani et al., 2007)
Prrx1	promotes EMT and invasion but suppresses metastatic colonization by inhibiting stemness	(Ocaña et al., 2012)
miR-200 s	inhibits EMT and invasion by suppressing ZEB1/2; promotes metastatic colonization by suppressing Sec23a-dependent secretion of metastasis inhibitory proteins	(Brabletz and Brabletz, 2010; Korpal et al., 2011)
miR-34 s	inhibits EMT and invasion by suppressing SNAIL1 translation	(Kim et al., 2011; Siemens et al., 2011)
ELF5	inhibits EMT and metastasis by suppressing SNAIL2	(Chakrabarti et al., 2012)
SIM2s	inhibits EMT and invasion by suppressing SNAIL2	(Laffin et al., 2008)
KLF17	inhibits EMT by suppressing ID1 expression	(Gumireddy et al., 2009)
CXCL1, IL6, IL-8, MMP1, Fascin-1	factors involved in tumor self-seeding	(Kim et al., 2009)
Survival in Circulation or Distant Organs		
TrkB	protects CTCs from anoikis by inducing AKT signaling	(Douma et al., 2004)
Wnt2	protects CTCs from anoikis by inducing FN1 expression through noncanonical Wnt signaling	(Yu et al., 2012)
Src	promotes survival of DTCs in bone by inducing AKT-dependent survival	(Zhang et al., 2009)
CXCL1/2	recruits $CD11b^+$ myeloid cells, which produce S100A8/9 to support tumor cell survival in metastasis and chemotherapy	(Acharyya et al., 2012)
VCAM1	recruits macrophage to support DTC survival by AKT signaling in lung metastasis; recruits and promotes osteoclastogenesis in bone metastasis; mediates immune evasion	(Chen et al., 2011; Lin et al., 2007; Lu et al., 2011)
IRF7	promotes immune escape of DTCs in bone metastasis	(Bidwell et al., 2012)
Metastasis Niches		
S100A8[a] and *S100A9*[a]	inflammatory proteins expressed by the lung, which recruit myeloid cells to establish the premetastatic niche	(Hiratsuka et al., 2006, 2008)
VEGFR1[a]	vascular endothelial growth factor receptor 1 (VEGFR1)+ bone marrow-derived hematopoietic progenitor cells form the premetastatic niche in lung metastasis	(Kaplan et al., 2005)
CCL5[a]	bone marrow-derived mesenchymal progenitor cells secrete CCL5, serving as chemoattractant to promote lung metastasis	(Karnoub et al., 2007)
TNC[a]	Tenascin C produced by tumor or stromal cells increases tumor stemness and promotes metastatic colonization	(Oskarsson et al., 2011)
POSTN[a]	tumor-associated fibroblast produces Periostin to maintain cancer stem cells by Wnt signaling	(Malanchi et al., 2012)
LOX1	recruits $CD11b^+$ myeloid cells to form premetastatic niche	(Erler et al., 2006, 2009)
Activation from Dormancy		
Coco	inhibits stroma-derived BMP signaling and enhancing cancer stem cell activities	(Gao et al., 2012b)
YAP	promotes cancer stem cell traits and metastatic outgrowth	(Chen et al., 2012; Cordenonsi et al., 2011)
β2AR[a]	activation of sympathetic neuron signaling increases receptor activator of NF-κB ligand production from osteoblast and promotes the formation of osteolytic metastasis	(Campbell et al., 2012)

[a]Genes are expressed by stromal cells.

associated with quiescence or reduced proliferation (Brabletz et al., 2001; Mejlvang et al., 2007; Vega et al., 2004), it has been hypothesized that disseminating cancer cells have to revert back to an epithelial phenotype (mesenchymal-epithelial transition [MET]) to establish a full-blown metastasis, which explains why metastases derived from carcinomas usually resemble their primary tumors and show an epithelial morphology (Brabletz et al., 2001). Indeed, the notion that MET may be required for effective colonization of DTCs in distant organs has been supported by recent experimental evidence in animal models (Chaffer et al., 2006; Korpal et al., 2011; Tsai et al., 2012; Tsuji et al., 2008). Thus, the current model is that tumor cells with a high degree of epithelial-mesenchymal plasticity are most likely to be the founder cells of metastases, because they can escape the primary tumor in a (semi)-mesenchymal and stem-like state and then establish a metastasis at the distant site by switching on their epithelial characteristics (Brabletz, 2012). Among the potential regulators of reversible EMT-MET transitions, the negative regulations of ZEB1/2 by miR-200 s, SNAIL1 by miR-34 s, SNAIL2 by transcriptional repressors Elf5 and SIM2s, and multiple EMT transcription factor by FBXL14/Ppa E3 ubiquitin ligase seems to be important (reviewed by De Craene and Berx, 2013).

Nevertheless, there are many open questions regarding the pathological relevance of EMT/MET switch in metastasis. At present, it is unknown whether CTCs with a (semi)-mesenchymal phenotype have to undergo MET in order to form metastasis and when/how this may happen at the cascade of metastasis. In breast cancer patients, DTCs in bone marrow express cytokeratins, and these epithelial DTCs are indicative of subsequent metastatic relapse (Braun et al., 2005). Thus, it can be postulated that MET might occur soon after extravasation of CTCs, when they are still at the single-cell stage. A recent study also showed that bone marrow-derived myeloid progenitor cells induce MET at the premetastatic niche and promote metastatic colonization (Gao et al., 2012a). To what extent the particular microenvironment of the secondary site might play a role as MET inducer or whether it is an intrinsic ability of tumor cells and whether DTCs from different cancer subtypes and in different metastatic organ sites have different degrees of plasticity to undergo MET is an important subject of future investigations. Therapeutic agents that inhibit epithelial-mesenchymal plasticity might be effective in reducing the risk of metastasis, particularly in high-risk time windows, such as surgery, which can produce a shower of DTCs.

Survival of Disseminated Tumor Cells in Secondary Organs

Understanding the mechanisms that allow latent CTC/DTCs to survive may be the key to the development of more effective adjuvant therapies (Uhr and Pantel, 2011). Recent studies have identified the AKT pathway as the major source of survival signals that can be activated by different context-dependent mechanisms. For example, TrkB was identified as a potent inhibitor of anoikis in detached tumor cells through activation of AKT (Douma et al., 2004). In breast cancer bone metastasis, the Src gene signature was linked with late-onset bone metastasis. Src mediates AKT regulation and the survival responses of cancer cells to proapoptotic signals of CXCL12 and TRAIL in the bone microenvironment (Zhang et al., 2009). In lung metastasis, VCAM-1 tethers tumor cells to macrophages and triggers AKT activation in cancer cells to protect them from TRAIL-induced apoptosis (Chen et al., 2011). VCAM-1 has also been shown to mediate immune evasion (Lin et al., 2007), which can also regulate the survival of DTCs (Bidwell et al., 2012). Interestingly, pAKT(S473) and AKT3-positive DTCs were detected in bone marrow samples of lung cancer patients and AKT1/AKT3-regulated proliferation, survival, migration, and epidermal growth factor (EGF)-mediated signal transduction of these DTCs (Grabinski et al., 2011). The development of technology platforms for genomic profiling of CTC/DTCs will further accelerate the identification of clinically relevant CTC/DTC survival pathways, as exemplified by the recent discovery of Wnt2-mediated noncanonical Wnt signaling in supporting the survival and metastasis of CTCs in pancreatic cancer (Yu et al., 2012).

An emerging concept in the study of DTCs is the potential existence of specific organ niches to enhance the survival of DTCs and protect them from microenvironmental or therapeutic stress. Recent experimental findings indicate that circulating prostate cancer cells can occupy the niches of hematopoietic stem cells after their arrival in the bone marrow (Shiozawa et al., 2011). These niches may keep the DTCs in a stem cell-like state and help them survive cellular stress and chemotherapy. Interestingly, the hematopoietic stem cell niches are located in the most hypoxic areas of the bone marrow, and adaptation to hypoxia might therefore be not only a driving force for cancer cells to leave the primary tumor but also a key process supporting the long-term survival of DTCs and formation of metastasis. Indeed, it has been shown that hypoxia induces stemness of cancer cells (Conley et al., 2012), stimulates the formation of premetastatic niche through recruitment of $CD11b^{+}$ myeloid cells by hypoxic tumor-derived LOX-1 (Erler et al., 2009), and promotes metastasis to bone and lung (Dunn et al., 2009; Lu et al., 2010). The common survival requirement under metastatic and chemotherapeutic conditions is also reflected in the recent discovery of the CXCL1/2 mediated recruitment of myeloid cells to produce chemokines S100A8/9 and enhance both metastasis and chemoresistance (Acharyya et al., 2012). It is notable that the formation of premetastatic niche in distant organs has been consistently shown to be induced by tumor-derived secretive factors or exosomes (Hiratsuka et al., 2006; Kaplan et al., 2005; Peinado et al., 2012), highlighting the importance of analyzing these factors in addition to DTC/CTC analysis in cancer patients. Such combined analysis may improve the effectiveness of using CTC/DTC as prognostic markers or therapeutic targets.

In addition to the contribution of recruited stromal cells to the survival of DTCs, the extracellular matrix has also been recognized as important components of the metastatic niche (Oskarsson and Massagué, 2012). For example, in lung metastasis, DTCs depends on Tenascin C (TNC), an extracellular matrix protein of stem cell niches, to maintain the metastasis-initiating capability of disseminated tumor (Oskarsson et al., 2011). The expression of Periostin (POSTN), another extracellular matrix component, is induced in tumor-associated stromal fibroblast and maintains cancer stem cells by activating Wnt signaling (Malanchi et al., 2012). Consistent with these experimental findings, positive TNC and POSTN staining were found to be an

independent poor prognosis marker in breast cancer patients (Oskarsson et al., 2011; Xu et al., 2012).

Breaking out of Dormancy

Significant insights have also been obtained from experimental and clinical studies regarding the outgrowth of DTCs or micrometastases, arguably the most rate-limiting step of the metastatic cascade (Cameron et al., 2000). There are many theories proposed to explain how DTCs are kept in a dormant or indolent state before the emergence of overt metastasis, including the lack of angiogenesis, immune surveillance, balanced proliferation and apoptosis, etc. (Uhr and Pantel, 2011). Bone marrow has been used as a model organ to study DTCs in breast cancer patients and repeated bone marrow sampling has revealed that DTCs were predominantly detected as single cells, whereas larger cell clusters indicating proliferative activity were rarely observed (Janni et al., 2011). These findings are consistent with previous reports demonstrating that more than 80% of patients with breast cancer (and other solid tumors) harbor only Ki67-negative, nonproliferating DTCs in their bone marrow (Pantel and Brakenhoff, 2004). These observations seem to challenge the proposed new model of parallel progression (Klein, 2009), which requires cellular proliferation of DTCs as a prerequisite for DTCs to progress to metastatic lesions independently from the primary tumor. Nevertheless, the bone marrow might have a particular dormancy-inducing environment and proliferating DTCs might exist in other secondary organs that are also preferential sites of metastases, such as liver, lung, or brain. It is also possible that some metastasis may arise from relatively small percentage of DTCs that only enters proliferative phase infrequently. Future rapid autopsy studies and genomic lineage analysis may provide more definitive clues to this important issue of metastatic progression (Steeg, 2008). Dormancy of DTCs may also be linked with the EMT state of the cells, as several previous studies showed reduced proliferation or quiescence of cells undergoing EMT, suggesting a requirement of MET for DTCs to resume proliferation as metastases (Brabletz, 2012).

A better understanding of dormancy may derive from the comparison of DTCs/CTCs in epithelial cancers with relatively short latencies, such as lung cancer, to those in breast cancer with long latency. Thus far, the information available in the literature is rather limited. Comparing the proliferative activity as measured by Ki67 immunostaining indicated that the fraction of Ki67-positive solitary CTCs is higher in lung cancer as compared to breast cancer (Hou et al., 2012; Müller et al., 2005), which is consistent with the different latency periods of both tumor types, and it may also explain why lung cancer CTC counts decline rapidly during chemotherapy (Hou et al., 2012), whereas breast cancer CTCs and DTCs appear to be more resistant to chemotherapy (Braun et al., 2000; Müller et al., 2005; Raimondi et al., 2011).

Another interesting comparison is colon cancer versus breast cancer with regards to DTCs in bone marrow. Although DTCs are detectable in this organ at similar frequencies in both tumor entities, overt bone metastases are rare in colon cancer but frequent in breast cancer (Pantel et al., 1993). Thus, colon DTCs are kept in a lifelong dormancy or they are eliminated. Indirect evidence that the immune system might control colon DTCs better than breast DTCs stems from phenotyping studies of DTCs, demonstrating that expression of histocompatibility leukocyte antigen class I molecules required for presenting tumor antigens to T cells is less frequently downregulated in colon DTCs than breast DTCs (Pantel et al., 1991).

Box 1. Open Questions about Cancer Dormancy

Do all cancer patients have dormant tumor cells?
Does EMT induce dormancy?
Do dormant cells have properties of cancer stem cells?
Are there preferred reservoirs of dormant cells (e.g., bone marrow)?
Can host factors, such as stress or inflammation, induce or break dormancy?
How does genetic background of the host affect dormancy of tumor cells in particular organs?
What signaling pathways or events reactivate dormant cells?
Does the immune system play a role in dormancy?
What is the effect of current therapies on dormant cells or dormancy?

Despite many outstanding questions regarding tumor dormancy (Box 1), development of animal models of metastatic dormancy has started to reveal organ-specific mechanisms of metastatic activation from dormancy. In bone metastasis, aberrant expression of VCAM-1 promotes the transition from indolent micrometastasis to overt metastasis by recruiting monocytic osteoclast progenitors and elevating local osteoclast activity to initiate the vicious cycle of osteolytic bone metastasis (Lu et al., 2011). In lung metastasis, Coco, a secreted antagonist of TGF-β ligands, induces solitary mammary carcinoma cells that have infiltrated the lung to exit from dormancy by blocking stroma-derived bone morphogenetic protein (BMP) signaling and enhancing cancer stem cell characteristics (Gao et al., 2012b). In other mouse models of dormancy, primary tumor is therapeutically resected to recapitulate the situation in cancer patients (Francia et al., 2011). Using such models, it is shown that the balance of p38α/β and ERK1/2 signaling affected cancer dormancy in breast and head and neck cancer (Sosa et al., 2011). Moreover, dormancy signatures differ between estrogen receptor (ER)+ and ER− breast cancer, with ER+ tumors having significantly higher dormancy signature scores, which is consistent with their prolonged dormancy before resuming metastatic growth 10 or more years after primary diagnosis (Kim et al., 2012).

Clinical Applications

Molecular mediators of tumor dissemination, survival, and outgrowth discussed above can be developed as biomarkers for diagnosis and prognosis as well as predictor markers for therapeutic agents that target these mediators (Alix-Panabières et al., 2012). Sophisticated technical platforms for the detection and molecular characterization of CTCs and DTCs have been established (Pantel et al., 2008). Currently, CTCs are included in more than 400 clinical trials as biomarker using various assays (Parkinson et al., 2012). The use of CTCs as "liquid biopsy" opens new avenues for genotyping/phenotyping of (micro)metastatic cells derived from various distant sites, which may present a more global picture than the biopsy of a single

metastasis at a particular site. In clinical practice, it is very difficult to obtain biopsies from multiple metastatic sites, whereas blood samples can be easily obtained during the course of cancer therapy, which allows a real-time monitoring of the pool of metastatic tumor cells. Thus, validating potential therapeutic candidate targets also through the analysis of CTCs will provide additional information, especially with regard to actual precursors of metastases in high-risk cancer patients. Interestingly, CTCs from individual patients have shown a striking heterogeneity in the status of some of these target genes and their downstream signaling pathways (e.g., HER-2, epidermal growth factor receptor, estrogen receptor, and androgen receptor) (Alix-Panabières and Pantel, 2013), suggesting that the selection of nonresponsive metastatic cells might be an important mechanism to escape targeted therapy. Recently, several interventional clinical studies based on the phenotyping of CTCs for therapeutic targets, such as HER-2, have been started (Bidard et al., 2012). CTCs compete with other blood-based biomarkers, in particular, circulating cell-free tumor DNA. Analysis of circulating DNA might also allow assessment of mutations relevant to dormant, residual, or recurrent disease and may guide treatment decisions in the future (Schwarzenbach et al., 2011). The analysis of cell-free DNA in blood serum or plasma samples offers the opportunity of easier storage and shipment compared to the CTC approach. However, tumor DNA in blood is diluted by variable amounts of normal DNA released by apoptotic cells, and sophisticated methods are, therefore, required to reveal tumor-specific genomic changes. Moreover, CTCs provide access to intact and viable metastatic tumor cells, which offers far more information than the analysis of degraded DNA released from apoptotic tumor cells, including the opportunity to use cultured CTCs for functional analyses. Thus, we believe that both approaches—CTC and circulating tumor DNA—are complementary as liquid biopsies.

Future Directions

The convergence of animal model and clinical studies of CTCs and DTCs has led to the development of an intellectual framework to guide future research and clinical development. However, many key questions remain to be addressed to translate better biological insights into improved therapeutics. Central among these is the lack of understanding regarding the distinguishing features between the vast majority of DTC/CTCs that are eliminated during different stages of dissemination or never escape dormancy versus those that eventually give rise to life-threatening metastases. Identifying the hallmark of high risk CTC/DTCs has the potential to revolutionize the design of clinical trials and facilitate the development of metastasis-preventing treatments. To functionally characterize CTC/DTCs from patients, protocols need be developed that allow cultivation and subsequent analysis of their tumorigenic-, stemness-, dormancy-, and metastasis-capacities, both in vitro and in vivo. Instead of relying on traditional trial endpoints, such as relapse-free survival or distant metastasis-free survival, using the reduction of high-risk CTCs as a surrogate but reliable endpoint can significantly shorten the time frame and thus reduce the cost of clinical trials for antimetastasis adjuvant therapies. Such improved clinical trial design may eventually lead to the approval of treatments that are highly effective in preventing metastasis but may have relatively minor effects in traditional trial endpoints, such as reducing tumor burden or improving the survival in late-stage patients.

Animal model studies will continue to identify potential biomarker, gene signatures, and functional mediators for the survival, activation, and expansion of metastasis from CTC/DTCs. Rapid improvements in single-cell genomic analysis of CTC/DTCs isolated from cancer patients will greatly facilitate the identification of important functional regulators of CTC/DTCs. Clinical validation of these new biomarkers/mediators requires meticulous collection and molecular analysis of DTC/CTCs throughout the course of disease progression in relevant patient cohorts. In addition to validating genes/proteins that can be clearly linked to the emergence of clinical metastasis, understanding the genetic and epigenetic basis for the activation of metastasis genes can provide valuable clues to the suspected connections of metastatic relapse to certain physiological or pathological conditions, such as stress, inflammation, infection, or hormonal changes. Such clinical observations will, in turn, inform the design of appropriate animal model experiments to vigorously test the hypothetical links.

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