Chapter 6
Circulating Tumor Cells and Tumor Dormancy

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Abstract Metastatic cancer can recur months or even years after apparently successful treatment of the primary tumor. While the exact mechanisms leading to cancer recurrence remain poorly understood, failure to completely eliminate dormant micrometastases and solitary metastatic cells is believed to be a major contributor. Thus, while not of initial clinical concern, metastatic dormancy is still a significant clinical problem. The emerging use of circulating tumor cells (CTCs) as prognostic and predictive biomarkers for monitoring and understanding metastatic disease may provide an opportunity to address this challenge. In this chapter we discuss the current knowledge relating to CTCs and tumor dormancy, and the relationship between the two with regard to metastasis biology and treatment. We also consider the clinical impact of monitoring for CTCs in the absence of symptomatic tumor recurrence and what is needed for such an approach to providing "actionable" information that will improve patient outcome.

Keywords Metastasis • Circulating tumor cells • Molecular characterization • Tumor dormancy • Oligometastasis

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6.1 Overview: Circulating Tumor Cells and Tumor Dormancy

Most cancer deaths are due to metastasis, which occurs when tumor cells spread from the primary tumor to establish themselves as secondary tumors in distant and vital organs, where they can cause physiological damage. Cancer that is diagnosed when it is localized to the primary site is easier to treat successfully, however most therapies eventually fail in the metastatic setting. Adjuvant therapy is given when there is a suspicion that the cancer has already seeded undiagnosed micrometastases, in order to prevent their subsequent growth, and has been shown to improve survival in many settings. Metastatic cancer can recur months or even years after apparently successful treatment of the primary tumor, and failure to completely eliminate dormant micrometastases and solitary metastatic cells is believed to be a major contributor to this recurrence. Thus, metastatic dormancy is a significant clinical problem.

Metastasis itself is a complex process, since the successful metastatic cell must traverse multiple steps in order to ultimately develop into a clinical relevant metastatic lesion. These steps include escape from the primary tumor, intravasation (invasion) into the lymphatic or hematogenous vasculature, survival in the circulation, arrest and extravastion into the secondary organ site, initiation of metastatic growth at that site, and maintenance of growth into macrometastases [1, 2]. Given the multistep nature of this process, there should be several opportunities for early identification of disseminating cells before they become a clinical problem. Indeed, in cancer patients with either metastatic or apparently localized disease, there is growing evidence that the presence of circulating tumor cells (CTCs) in the blood is an important indicator of metastasis and poor outcome (reviewed in Refs. [3, 4]). The emerging use of CTCs as prognostic and predictive biomarkers for monitoring and understanding metastatic disease may provide an opportunity to address the challenge of metastatic dormancy. In this chapter we discuss the current knowledge relating to CTCs and tumor dormancy, and the relationship between the two with regard to metastasis biology and treatment.

6.2 Circulating Tumor Cells

Although CTCs have been recognized for well over a century [5], only recently has technological advancement allowed for detailed investigation of these rare cells and their consideration for use in the clinic. Even in patients with known metastatic disease, these cells are present at a very low frequency in the circulation (~1 CTC per 10^5–10^7 leukocytes) [6–8], necessitating the development of sensitive and specific approaches for their isolation, enumeration and molecular characterization. The enormous promise of CTCs for monitoring disease recurrence and treatment response in clinical oncology has resulted in an explosion of interest in developing biomarker

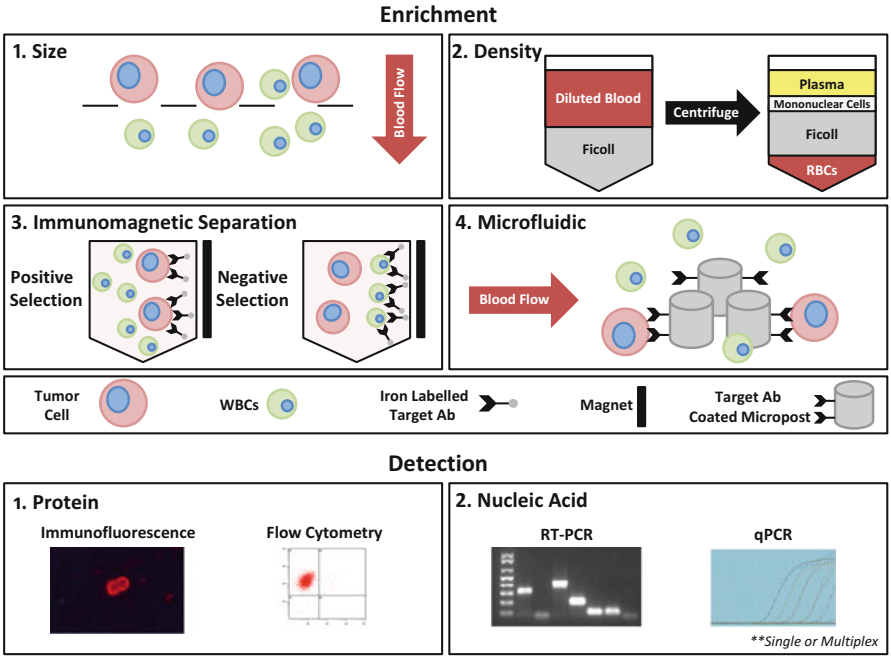

Fig. 6.1 An overview of the most commonly utilized techniques for the process of CTC enrichment and detection. In general, four approaches currently exist for CTC enrichment (*1*) size-based; (*2*) density-based; (*3*) immunomagnetic separation; and (*4*) microfluidic-based. Using size-based enrichment techniques, diluted whole blood is passed through a filtration device with specific sized pores (typically 8 μm). CTCs are captured based on differences in cell size between CTCs (typically >8 μm) and white blood cells (WBCs; typically <8 μm). Density-based enrichment utilizes Ficoll (or similar density gradient medium) to enrich for mononuclear cells (including CTCs) from other blood components. Immunomagnetic separation involves the use of iron-conjugated antibodies targeted toward CTCs (e.g., EpCAM; positive selection) or contaminating blood cells (e.g., CD45; negative selection) and incubation in a magnetic field. For microfluidic-based techniques, whole blood is slowly passed across a chip-based surface and isolated using either CTC targeted antibody-coated microposts (CTC Chip and iChip), or dielectrophoresis (DEPArray). Current CTC detection techniques use either a protein-based approach (i.e., immunofluorescence or flow cytometry) expressed by whole cells or secreted proteins (EPISPOT assay), or nucleic acid-based approaches such as RT-PCR or RT-qPCR, applied at the level of single genes or using a multiplex approach. *Re-printed from Lowes LE, Allan AL. Recent advances in the molecular characterization of circulating tumor cells. Cancers (Basel). 2014 Mar 13;6(1):595-624. doi: 10.3390/cancers6010595 (Open Access)*

approaches for analyzing CTCs in the clinical setting as well as understanding the underlying biology of these cells and their functional relationship to metastasis and tumor dormancy. As a result, more than 40 different CTC technologies are currently under development [9], many of which are described in greater detail elsewhere in this book. However, common themes are emerging that help to categorize these technologies into some key approaches that are required for successful enrichment, isolation, detection, and/or molecular characterization of CTCs (Fig. 6.1).

For enrichment, approaches include size-based, density-based, or immunomagnetic enrichment (i.e., positive selection of CTCs using epithelial-specific or tumor-specific markers, or negative selection using markers expressed by contaminating cells such as leukocytes). For detection and characterization, approaches include cytometric techniques such as immunofluorescence or flow cytometry using antibody-mediated detection; or nucleic acid-based techniques such as reverse transcription polymerase chain reaction (RT-PCR), quantitative-PCR (qPCR), microarray, or sequencing. The advantages and disadvantages of each of these approaches have been extensively reviewed elsewhere [9–12] and therefore not discussed in detail here.

6.2.1 Clinical Utility of CTCs

The intense interest in CTCs is evidenced by the fact that more than 400 clinical trials have or are utilizing CTCs as correlative biomarkers, and PubMed lists more than 14,000 publications involving CTCs [13]. However, despite the number and scope of these studies, the CellSearch® system (Janssen Diagnostics) remains the only CTC platform presently cleared by the FDA for detection and enumeration of CTCs in the clinical setting. This platform enriches for CTCs using positive immunomagnetic selection based on EpCAM (epithelial cell adhesion molecule) expression, followed by immunofluorescent staining for cytokeratins (CK 8/18/19), CD45 (to identify contaminating leukocytes), and the DNA dye DAPI (4′,6-diamidino-2-phenylindole). Using semi-automated fluorescence microscopy, positive CTCs are then identified as cells >4 μm in diameter with an intact cell membrane and a CK+/DAPI+/CD45− phenotype [14]. The CellSearch® system is currently FDA-cleared for prognostic use in metastatic breast, prostate, and colorectal cancers, where the presence of ≥5 (breast [14] and prostate [15]) or ≥3 (colorectal [16]) CTCs in 7.5 mL of blood has been correlated with poorer prognosis compared to patients with fewer CTCs in the same blood volume. Using this platform, CTC enumeration has been utilized not only to assess CTC number at baseline but also for serial assessment of response to treatment. It has been demonstrated that CTCs are correlated with patient outcome and that a change in CTC number during treatment is predictive of therapy response, often sooner than currently utilized techniques such as imaging [15, 17–19].

Thus far the greatest clinical utility for CTCs has been observed in the metastatic setting for breast and prostate cancer, with growing evidence in colorectal and lung cancer [14–16, 20–23]. In addition, the amount of CTC data available in the literature has facilitated several meta-analyses that have highlighted the prognostic value of CTCs in various cancers, including pancreatic [24], lung [23], colorectal [25], breast [26], and prostate cancer [27]. In particular, Zhang et al. (2012) analyzed data from thousands of breast cancer patients and demonstrated that CTCs are a stable prognosticator in both metastatic and early-stage breast cancer [26]. Importantly, CTCs are now taken into account in the American Joint Committee on

Cancer (AJCC) TNM (tumor-node-metastasis) cancer staging manual for breast cancer; as classification cM_0(i+) ("No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumor cells in circulating blood, bone marrow, or other nonregional nodal tissue that are ≤0.2 mm in a patient without symptoms or signs of metastases") [28]. Taken together, this body of clinical data provides convincing evidence to support the use of CTCs for tracking, understanding and treating metastatic disease in cancer patients.

6.2.2 Challenges and Potential of CTC Analysis

Several challenges still exist in the analysis of CTCs, including technological and statistical challenges (sensitivity, specificity, reproducibility, capacity for single-cell molecular characterization); biophysical parameters (clustering of CTCs with each other and/or with leukocytes and platelets, reduced capture of CTCs due to size restriction in small capillary beds); and biological factors (immune surveillance, loss of epithelial phenotype through epithelial–mesenchymal transition (EMT), molecular and cellular heterogeneity between individual CTCs in the same patient, and the relationship with cancer stem cells) (reviewed in Refs. [13, 29, 30]). These challenges are highlighted by the fact that although CTCs are useful in many epithelial cancer types discussed above, they have been found to be only minimally informative in other cancer types, either because of the biology of how the disease progresses/metastasizes (i.e., localized versus distant dissemination in cancers such as ovarian, liver, and brain cancer) and/or the lack of expression of epithelial markers which may impact the ability to detect CTCs from these cancers by most current clinical CTC approaches (i.e., renal cancer). It is also important to note that current CTC technologies are not always sensitive enough to reproducibly detect the lower numbers of CTCs that may be present in patients with early-stage disease or those in the adjuvant setting, where the risk of recurrence or metastasis is unknown [6, 29].

Although the CellSearch® system is considered the current "gold standard" for clinical CTC enumeration, its sensitivity and capacity for downstream molecular analysis is limited [6, 9]. Emerging technologies such as Epic Sciences' HD-CTC fluid biopsy (developed by Peter Kuhn and colleagues) [31–34], and the CTC Chip/iChip (developed by Mehmet Toner, Daniel Haber and colleagues) [20, 35–42] have shown great promise with regards to reproducibility in a clinical lab setting and have the added advantage of increased sensitivity for assessing earlier stage disease as well as the capacity for molecular characterization of CTCs. The HD-CTC fluid biopsy assay identifies CTCs without using surface protein-based enrichment, instead using sophisticated imaging and software algorithms to identify and present CTCs as high definition (HD) diagnostic pathology quality images [31–34]. The most developed version of the CTC Chip (the iChip) also does not rely on the presence of EpCAM or other known tumor antigens on the cell surface of CTCs, taking the approach of combining microfluidics with sequential negative and positive

enrichment methods on a herringbone microchip [37]. Both assays (HD-CTC and iChip) have been demonstrated to have improved sensitivity over the CellSearch® system and can provide CTCs in an ideal format for downstream characterization using various approaches including fluorescence in situ hybridization (FISH), immunofluorescence and mutational analysis.

6.3 Molecular Characterization of CTCs

Given our increasing awareness of tumor heterogeneity and the ability of tumors to evolve at the molecular level during disease progression, it is becoming apparent that simple enumeration of CTCs fails to capitalize on their full potential as biomarkers of metastatic disease. Perhaps the greatest promise that CTCs hold for oncology lies at the level of molecular characterization. Given the fact that metastasis determines the ultimate outcome for a patient, treatment decisions may be more effective if they are based on the genetic characteristics of metastatic lesions rather than on those of the primary tumor alone. However, obtaining biopsies from metastatic tumor tissue is an expensive, invasive and often painful procedure, limiting its widespread use in clinical practice [43]. Since CTCs are the intermediaries between primary and metastatic disease and are believed to be surrogates of a patient's metastatic tumor [44–46], molecular characterization of CTCs may provide an opportunity for noninvasive "real-time" biopsies during disease progression in order to track these molecular changes and potentially incorporate them into clinical decision making.

Several studies suggest that molecular characterization of CTCs may have clinical utility from the perspective of identifying loss or acquisition of molecular features in individual patients' tumors that may open up new avenues for targeted therapy that were not options based on the characteristics of the primary tumor alone. For example, Meng et al. demonstrated that almost 40 % of metastatic breast cancer patients who were initially HER2-negative (based on their primary tumor) acquired amplification of HER2 in their CTCs. When treated with Herceptin based on CTC HER2 amplification, some of these patients demonstrated a partial or complete response [47]. Several other subsequent studies have demonstrated discordance between HER2 status in patients' CTCs versus their primary tumor [48–53] and demonstrated that HER2-positive CTCs are a poor prognostic factor in patients with both early-stage and metastatic breast cancer [53–55]. Similar studies in prostate cancer have demonstrates an evolution in important disease-related markers such as AR, PTEN, and TMPRESS2ERG between primary tumors and CTCs that may help identify those patients most likely to respond (or not) to targeted therapies [35, 56–59].

In addition to examining individual molecular markers on CTCs that may have prognostic or predictive value, the CTC field has also benefited from technological advances in more sophisticated downstream analysis approaches such as genomic

sequencing. Recent studies have reported isolation and analysis of genomic DNA from CTCs and single cell analysis of copy number variation patterns, array-CGH, and next-generation sequencing [60–62]. Ni et al. (2013) analyzed single CTCs from lung cancer patients and observed that every CTC from an individual patient exhibited reproducible copy number variation (CNV) patterns, similar to those of the metastases (but not primary tumor) of the same patient [61], supporting the idea that CTCs can serve as a reflection of the molecular features of metastatic disease. Another study by Heitzer et al. (2013) observed that in CTCs from colorectal cancer patients, mutations in known driver genes such as APC, PIC3CA, and KRAS could be found in matched primary tumors, metastases, and CTCs; however mutations exclusive to CTCs were also observed. Interestingly, additional deep sequencing of tumor tissue demonstrated that most mutations initially found only in CTCs were actually present at a subclonal level in the primary tumors and metastases from the same patient [60], suggesting that CTCs are representative of the complex and heterogeneous tumor genome.

Although large-scale clinical data is still lacking with regards to the value of molecular characterization of CTCs as a clinical decision making tool, the studies described above suggest that this type of analysis holds tremendous promise with regards to developing personalized approaches to therapy and providing valuable insight into the underlying biology of metastasis and tumor dormancy.

6.4 Implications of CTCs for Understanding Metastasis Biology

In contrast to most areas of cancer research, the study of CTCs began in the clinic rather than at the laboratory bench. As a result, the majority of CTC studies have focused on technology development and clinical utility, with minimal investigation into the biology of CTCs until fairly recently. Initial CTC work in experimental systems utilized immortalized human cancer cell lines and xenograft mouse models, demonstrating that CTCs can be serially tracked over time in preclinical models and that increasing numbers of CTCs are correlated with increased metastatic burden [10, 46, 63, 64]. Additional studies in both preclinical models and patient samples have started to define some of the mechanisms underlying CTC progression to metastases, including hypoxia [65, 66], epithelial–mesenchymal transition (EMT) [41, 67–73], and stem cell-related signaling [42, 44, 69, 71, 74]. Importantly, it has now been demonstrated that viable CTCs can be isolated from patients and grown in culture [75–77] as well as injected into immunocompromised mice to initiate metastases [44, 77]. These studies highlight the critical role that CTCs play in disease progression and metastases development, and open up exciting possibilities for future experimental and clinical studies aimed at interrogating the role of CTCs in tumor dormancy.

6.5 Tumor Dormancy

6.5.1 Clinical Tumor Dormancy

One of the most problematic aspects of cancer is its ability to recur after apparently successful primary treatment. In some cases, these recurrences can be years or even decades after initial diagnosis and treatment. While some patients with melanoma, kidney cancer and breast cancer are often believed to be at particular risk for late recurrences, these can occur at low frequency for many cancer types; see Table 1 in Ref. [78]. Uncertainty about which patients will have late recurrences makes ongoing care of these patients difficult. Several clinical trials, including the MA.17 trial of long-term hormonal treatment in women with hormone responsive breast cancer, have indicated that tumor dormancy and late recurrences are a clinical reality, and that long-term therapy does offer some benefit in preventing micrometastatic disease from progressing [79–81]. However, these recurrences occur in a relatively small proportion of these patients, and this benefit to the group needs to be weighed against the toxicities associated with long term therapies for many patients [78, 82, 83].

6.5.2 "Cure" vs. "Clinical Dormancy"?

A dilemma about tumor dormancy and late recurrence is illustrated in Fig. 6.2. Figure 6.2a depicts the clinical situation following apparently successful treatment of a primary cancer. It is not known whether this patient has truly been cured, or if there is undiagnosed, micrometastatic but dormant disease present that will (or may?) recur. It is only after a cancer does recur that the patient can be categorized as having had dormant cancer that subsequently began to grow (Fig. 6.2b). Thus, a clinical identification/diagnosis of "dormancy" currently can only be made after the fact of recurrence. However, evolving technologies for monitoring for evidence of dormancy and micrometastatic disease, including CTCs and other blood biomarkers, as well as improving imaging approaches for detection of minimal residual disease may lead to an improved ability to detect small volumes of residual cancer. This information will then need to be appropriately integrated into cancer management strategies, discussed below.

6.6 Biology of Clinical Tumor Dormancy

Much more needs to be learned about the biology of tumor dormancy, which patients are at risk for dormancy and recurrences, and also whether micrometastatic disease is destined to recur or if there are lifestyle or therapeutic/preventive interventions that can

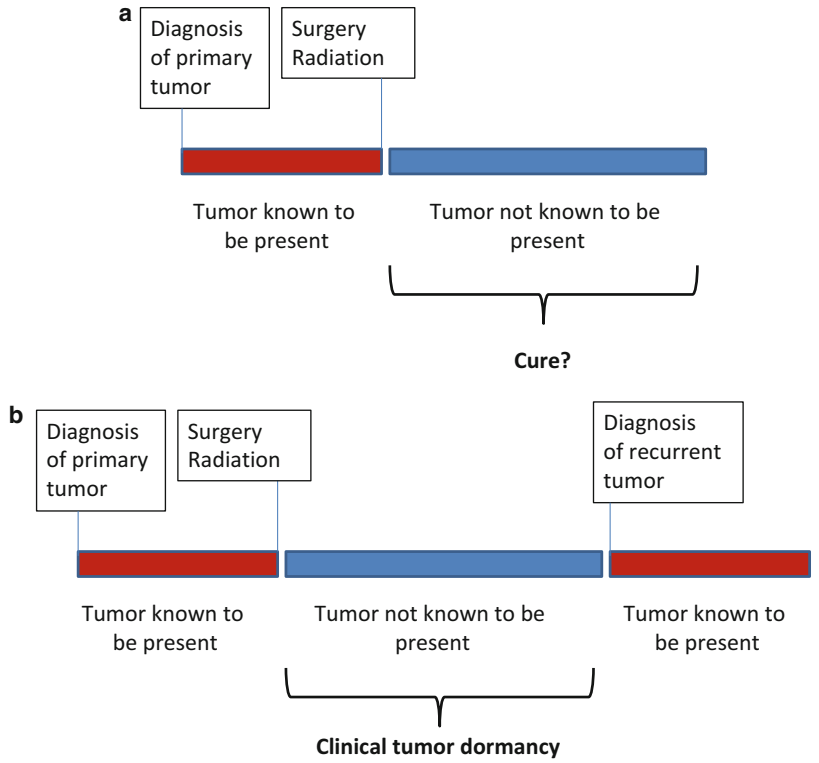

Fig. 6.2 Clinical tumor dormancy can be defined only after the fact of tumor recurrence. (**a**) Depicts the situation of a patient who was diagnosed with cancer and received local therapy (surgery, radiation) and perhaps adjuvant systemic therapy designed to eliminate any disseminated cancer. The *red bar* depicts the clinical situation of cancer known to be present. The *blue bar* depicts the situation of cancer not known to be present. The dilemma is that it is not known if this patient is cancer-free and cured, or if undiagnosed cancer still remains. If the *blue bar* represents a "long" period of time (e.g., 5 years, or more), the patient might be considered to be "cured." (**b**) Depicts the situation of a patient whose cancer recurs. It is only after cancer recurrence that it can be known that undetected cancer was present, and if the *blue bar* represents a "long" period of time, that the cancer was indeed persistent but in a state of "clinical dormancy." This diagram has no implications of mechanisms of maintenance of dormancy, location or state of cancer cells in this interval, or causes of cancer recurrence, which remain important research questions

minimize recurrence. Progress toward better understanding of the biology of tumor dormancy has come from both experimental and clinical studies (many focused on breast cancer), which in turn have led to hypotheses about clinical tumor dormancy.

Early case observations and thoughts on clinical tumor dormancy have been reviewed by Meltzer in 1990 [84]. Discussions about the possible kinetics of tumor growth and dormancy have been presented by Demichelli and colleagues, who concluded that clinical tumor dormancy likely is a consequence of arrested and restarted growth, rather than very slow, continuous growth [85]. A recent review by Uhr and Pantel concludes that "Clinical data suggest that a majority of breast cancer survivors

have cancer cells for decades but can remain clinically cancer-free for their lifetime" [86]. Clearly, the identification of molecular mechanisms responsible for this natural, long-term cancer control in patients, inherent to the state of clinical tumor dormancy, will be important to understand and may lead to interventions to maintain or prolong dormancy therapeutically

6.7 Experimental Models of Tumor Dormancy

A variety of in vivo and in vitro models have been used to try to understand the biology underlying tumor dormancy. Tumor dormancy has been observed in experimental metastasis models from many cancer types, reviewed in Refs. [87–89]. Experimental dormancy has been described as both cancer cell quiescence, evidenced by solitary cancer cells that persist in vivo without cell division [90, 91], and as pre-angiogenic micrometastases with balanced cell division and apoptosis, such that there is no net growth [92, 93]. When mice are treated with cytotoxic chemotherapy that targets dividing cells, cancer cells that are in quiescent state at the time of treatment have been shown to be insensitive to this therapy, leading to subsequent late recurrences [94–96]. These studies are consistent with clinical late recurrences, which can occur in some patients following adjuvant chemotherapy [1].

Many studies have attempted to decipher the molecular mechanisms that can regulate tumor dormancy. Some common themes have emerged, including the ability of the microenvironment surrounding a cancer cell in secondary sites to influence entry into, or maintenance of, a dormant state [97–100], as well as properties of the cancer cells themselves, such as expression of metastasis suppressor genes, reviews [101, 102]. A few examples of studies supporting these mechanisms of regulation of dormancy are presented below, and the articles and reviews cited above provide more examples.

Early studies by Ossowski and Aguirre-Ghiso, using a chicken embryo in vivo model, showed that reduction of urokinase plasminogen activator, limited β1 integrin activity and consequent reduction in interactions with the extracellular matrix, were associated with dormant cancer cell behavior [103–106]. Barkan et al. [107] adapted assays developed by Bissell and colleagues [108], in which cells are grown in 3D Matrigel matrices. Using these assays, Barken et al. found that multiple cancer cell lines that showed prolonged dormant behavior versus active metastatic growth in experimental mice also showed parallel "dormant" versus "proliferative" behavior in vitro. This in vitro assay thus enabled identification of molecular properties that reflected these growth patterns. Dormant cells showed cell cycle arrest and nuclear expression of the cell cycle regulators p16 and p27. In contrast, cells that made the transition from quiescence to proliferation increased production of fibronectin and β1 integrin signaling, cytoskeletal filamentous actin stress fiber formation and phosphorylation of myosin light chain (MLC) via MLC kinase. This study also showed that inhibition of inhibition of β1 integrin or MLCK prevented the transition from dormancy to proliferation, suggesting that regulation of interactions between cancer

cells and the extracellular matrix may provide a therapeutic target to regulate tumor dormancy [100, 107, 109]. Other studies have also implicated regulation of cancer cell–microenvironment interactions in maintenance of tumor dormancy [110]. For example, recent work by Bissell and colleagues suggests that, in mouse models, that mature blood vessels can suppress metastatic outgrowth, whereas sprouting microvasculature may induce a proliferative phenotype in cancer cells [111].

While great progress thus has been made in clarifying how dormancy and proliferation may be regulated experimentally, much remains to be learned about molecular mechanisms that can regulate dormancy and, importantly, how then to translate these experimental findings to achieve regulation of tumor dormancy clinically. The ways in which CTCs may provide information about dormancy are discussed below.

6.8 Relationship Between CTCs and Clinical Dormancy

Meng et al. [112] observed that CTCs could be detected in breast cancer patients free of overt metastases up to 22 years after their initial diagnosis, suggesting that many apparently "cured" cancer patients may harbor detectable dormant tumor cells [112]. However, the clinical implications of these findings remain unclear. Although very few studies have definitively shown that CTCs can provide reliable evidence of occult metastases, minimal residual disease, and/or clinical dormancy, exciting advances in CTC technology provide promise for understanding the relationship between CTCs and clinical dormancy. The benefit of early detection of metastases depends on whether it is possible to distinguish those cancers that are likely to behave aggressively from those that are indolent, and/or will never leave their state of dormancy. To achieve this, one might envision that molecular characterization of CTCs using a "dormancy versus proliferation" expression signature (i.e., Ki67, MLCK, β1 integrin, fibronectin, p16, p27) and correlation with time to metastatic recurrence in patients may provide critical insight into how CTCs are related to dormancy. In addition, the recent demonstration that viable CTCs can be isolated from patients and grown in culture [75–77] as well as injected into immunocompromised mice to initiate metastases [44, 77], may facilitate the study of functional behavior of dormant versus proliferative CTCs derived from patients as well as the underlying mechanisms of this behavior in greater detail than has been previously possible. Finally, molecular and functional comparison of CTC characteristics in epithelial tumor types with relatively short latency periods (i.e., lung or colorectal cancer) to those cancers with long periods of latency (i.e., breast cancer) may provide a greater understanding of the relationship between CTCs and clinical dormancy [88].

Since monitoring for CTCs is relatively noninvasive, requiring only a blood sample, this approach would have advantages over more invasive approaches such as molecular imaging. However, at this stage it is not known whether detection of CTCs as an early signal of cancer recurrence will lead to patient benefit, and it is unclear whether cancers detected by presence of increases in CTCs are more readily

treated than cancer recurrence based on current clinical practice. The presence of CTCs in cancer survivors who have no other evidence of persistent disease indicates that these individuals have dormant cancer somewhere in their bodies, which are shedding and replenishing the CTC population. Biologically, this evidence of persistent but asymptomatic disease offers many important opportunities to learn about cancer metastasis and dormancy, including how their progressive growth is being controlled. This information could provide new approaches for cancer control, through maintenance of the dormant state and/or through improved knowledge about how to eliminate dormant cancer.

6.9 Conclusions and Future Perspectives

Several cancer types can recur after long periods of an apparent cancer-free state. Analysis of CTCs may have potential value for monitoring patients at risk for cancer recurrence, especially delayed recurrence. Clinically, however, knowledge of CTCs in otherwise healthy individuals may lead to treatment dilemmas and harm, or lack of clinical benefit, to these people. Cancer that has not spread to distant sites is often treated with curative intent, whereas distant metastatic disease is generally regarded as not curable, at least with currently available therapies. The question of whether early detection of metastatic, recurrent disease offers clinical benefit to patients is an important but unanswered question. Thus, earlier detection of metastatic disease may lead to earlier treatment of asymptomatic individuals with a limited number of anti-metastatic therapies, with the inherent toxicities of these agents and without demonstrated knowledge that "early" metastatic disease is more treatable than later, symptomatic metastatic disease. Recently, Jochelson et al. discussed this issue in the context of breast cancer, and concluded that there is no evidence that, for women with recurrent breast cancer, earlier detection has clinical benefit to the patient [113].

One possible exception to this concern may lie in the case of oligometastatic disease, sometimes defined as few (e.g., 1–3) metastases confined to a single secondary site. While the subject of ongoing debate, in some cases, treatment of oligometastases with local treatment, such as surgery or stereotactic radiation, has been shown to lead to long-term benefit for some patients [114]. In other cases, however, local oligometastatic therapy may initially be successful, only to have additional metastases become apparent; presumably these were present as additional, undetectable micrometastases at the time of diagnosis and treatment of the oligometastases. Whether metastases detected "early" are less-progressed, and more treatable, than those detected later remains an unanswered question.

In conclusion, when evidence of persistent cancer is detected using CTCs or other circulating biomarkers, then clinical utility of this knowledge will depend on the reliability of these biomarkers to detect cancer, as well as the availability of clinically beneficial therapies for patients in that setting. Biologically, monitoring patients for recurrent, metastatic disease will be important in our understanding of

metastatic disease and dormancy, and this knowledge may in future lead to improved clinical management. Metastatic disease, for breast and most other cancers, is generally considered to be ultimately incurable, so clinical implementation of CTCs or other biomarkers to monitor for tumor recurrence will vary with the specifics of the particular cancer. However, the wealth of biological information that may be gleaned from serially analyzing the presence and fluctuation of CTCs over time, as well as (and perhaps more importantly) their molecular and functional characteristics may offer the opportunity to lead to clinical benefit for patients with persistent, dormant cancer.

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