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Circulating Tumor Cells: Strategies for Capture, Analyses, and Propagation

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Abstract

Circulating tumor cells (CTC) play a central role in tumor dissemination and metastases, which are ultimately responsible for most cancer deaths. Technologies that allow for identification and enumeration of rare CTC from cancer patients' blood have already established CTC as an important clinical biomarker for cancer diagnosis and prognosis. Indeed, current efforts to robustly characterize CTC as well as the associated cells of the tumor microenvironment such as circulating cancer associated fibroblasts (cCAF), are poised to unmask key insights into the metastatic process. Ultimately, the clinical utility of CTC will be fully realized once CTC can be reliably cultured and proliferated as a biospecimen for precision management of cancer patients, and for discovery of novel therapeutics. In this review, we highlight the latest CTC capture and analyses technologies, and discuss *in vitro* strategies for culturing and propagating CTC.

Keywords

Circulating tumor cells; CTC; clinical biomarkers; cCAF

Cancer remains among the most common causes of morbidity and mortality worldwide. Although screening methods and better systemic treatment have led to a decrease in cancer related mortality, patients with metastatic disease mostly remain incurable. With development of novel systemic treatment it may even become more crucial to detect early occult metastatic spread. While the phenomenon of lymphatic tumor spread is independent,

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and its clinical and prognostic significance a matter of long debate which is out of the current scope, hematogeneous spread is associated with occurrence of distance metastases. The focus of detection of occult tumor spread has been recently on peripheral blood, as opposite to previous studies on bone marrow dissemination. In peripheral blood either tumor cells (circulating tumor cells, CTC) or circulating tumor associated nucleic acids, (DNA, RNA, and of special importance microRNA) are detected. Latest capture platforms are reviewed in the next section, and placed in the light of technology improvements needed to detect and enumerate CTC.

CTC capture technologies

The greatest challenge for CTC detection in peripheral blood is their rarity. Only few CTC are detected even in patients with advanced metastatic disease. The frequency of CTC is often less than one CTC per ml of peripheral blood. Therefore, a variety of techniques for CTC enrichment has been established. These technologies make use of either biological and/or physical properties of tumor cells that help distinguish CTC from all the normal blood cells[1–3]. We have reviewed most common technologies elsewhere and here we provide only a brief summary[4].

Affinity-based methods take advantage of antigens that are differentially expressed by CTC (positive enrichment, EpCAM is mostly used)[5,6], or by blood cells (negative selection, e.g. CD45,)[7,8]. Most commonly, magnetic beads are armed with antibodies for positive or negative separation[9]. Also columns or cartridges can be used[5,10] and most recently microchips have been coated with antibodies[6,11]. Through this methodology, only a subset of the CTCs are captured from the patient sample, namely the EpCAM positive cells[12,13]. However, as tumor cells exhibit heterogeneity and thus there is high variability of expression, resulting in some tumor cells having no or very low expression of EpCAM they evade capture[14–18]. Further, EpCAM being an epithelial cell biomarker, limits the ability to capture CTC from epithelial tumors that show low or no EpCAM expression (e.g. renal cancer), non-epithelial tumors such as melanomas and sarcomas, or CTC that have undergone epithelial-to-mesenchymal (EMT) transition to form cancer stem cells (CSC)[13,19–21].Technologies in this category also work through the reverse process of using CD45 antibody based depletion of white blood cell to leave behind the CTC. Further hindrances are experienced when these platforms are microfluidic based systems as they have limitations on sample volumes they are able to process. And the small volumes that are processed require extended processing times; such as the CTC-iChip can process 8mL of whole blood/hour with and an additional 1 hour set-up time, thus only 8mL can be processed over a period of 2 hours[22]. It is important to note though, that there is only one FDA approved platform on the market for CTC capture at this time, which is CellSearch ® a technology based on magnetic EpCAM Ab based separation[4,23].

Differences in cell density can be also used for enrichment, and the best known method based on density is Ficoll Hypaque separation[24], which separates red blood cells from nucleated cells and tumor cells remain with the nucleated cells. As alternative property of tumor cells for enrichment, cell size is being used[25], based on the fact that tumor cells are larger than most blood cells[26]. We have developed a size-based microfilter for enrichment

and detection of CTC[27] (Figure 1), which is highly efficient and faster than affinity-based separation techniques and can be used for a wide range of molecular applications for additional characterization of CTCs[28]. This microfilter platform has the advantage of capturing CTC from a wide variety (nearly all) solid tumors (Figure 1D-I), including non-epithelial, non-EMA expressing cells such as melanoma (Figure 1I). In addition, we have developed the ability to efficiently capture live cells (Figure 1B), and release fixed and live cells (Figure 1C), which makes possible a wide variety of downstream applications, including CTC propagation.

After enrichment, a variety of technologies can be used to distinguish CTC from the nonspecifically captured cells including cytomorphological characterization of CTC, immunohistochemical/immunofluorescent (IHC/IF) detection of tumor specific antigens, or various real time polymerase chain reaction (RT-PCR) approaches. Immunocytochemical detection of CTC relies on antibody based detection of cells using antibodies specific for epithelial cells. Most commonly used antibodies are cytokeratins[29]. It is now often combined with markers such as CD45 that identify the background blood (non-CTC) cells. Multiplex IHC/IF approaches enable simultaneous visualization of multiple markers on a single cell. Molecular characterization of CTC is carried out by various strategies that include fluorescent in situ hybridization (FISH), comparative genomic hybridization (CGH), PCR-based techniques, RNA-seq, and immunofluorescence. These studies have shed light on the oncogenic profile and metastatic potential of CTC and have allowed the comparison of the genetic profile of tumor metastases and CTC to that of their primary tumor counterpart.

Gaps in imaging and image acquisition

More fundamentally, imaging based commercial technologies are still tied to the traditional microscopy imaging approach and as such still suffer from the same limitations that traditional microscopy has. These limitations are significant and ill-matched to the demands that CTC analysis imposes. First, traditional microscopy is highly susceptible to out-of-focus issues[30]. Well focused images are required for high quality downstream image analysis, which can only be achieved manually or through sophisticated optical and focusing instrumentation. The problem is further compounded if the sample of interest is presented on a platform that has even minor uneven height-varying distribution. In such a case, it would be impossible for a single microscopy snapshot to display all parts of the sample in focus. Z-stacking, whereby multiple microscopy images taken at a sequence of focal plane, would be required in traditional microscopy leading to increased image acquisition times and a much increase data volume. The Fourier Ptychographic Microscopy (FPM) approach (Figure 2) addresses this issue as it uniquely enables post-image acquisition refocusing[31]. Second, it is desirable to view images using different light sources, phase contrast, bright-field and fluorescent. For example, phase contrast image data is very useful in helping to delineate cell boundaries and enhance the visibility of internal organelles, and fluorescent data is used to establish marker status and distinguish cell types; these different light sources generally require switching in a different set of optics into a traditional microscope to acquire the image. The FPM addresses this gab by providing this data automatically without additional optics[31], thereby allowing fluorescence, bright field and phase imaging to be performed without swapping of optics. Finally, a traditional microscope is bounded in the number of

resolvable pixel information it can deliver – if a user wants high resolution, the field-of-view would have to commensurately decrease, and vice versa. This relationship is fundamentally tied to the physical optics aberration limitations. This image information bound forces most wide field of view imaging system to perform mechanical image scanning, which leads to a host of engineering challenges and difficulties, such as scan uniformity, vibrational control and scan speed limitations. The FPM approach breaks this bound – in prior work, Yang et al. have demonstrated FPM imaging system that provides orders of magnitude more resolvable pixel information through the same optical column[32]. FPM can perform wide field of view imaging without mechanical actuations.

Given the body of evidence that CTC hold prognostic value, properties of an optimal capture and analysis platform are clear. It is clearly desirable to achieve a high capture efficiency without use of affinity tags. The technology needs to be robust, efficient and able to accommodate the ability to capture fixed cells, live cells, clusters as well as TME cells such as CAFs. The platform should be able to accommodate a variety of blood volumes, including large volumes that may be important in evaluating patients with early stage cancer. While the evaluation of cells depends on visualization of cells and their targets for CTC/liquid biopsy to be a clinical test, there will be a requirement to incorporate robust and high-quality image acquisition and automated image analysis that is capable of characterizing complex cell features, including morphology and target expression. In addition, there is a necessity to have the platform in a form factor that allows implementation into a reference lab setting, requiring minimal user interaction while having medium to high throughput capabilities.

Global Genomic Profiling of CTC

The greatest advances in the treatment of cancer have been made with combinations of targeted therapies. The identification of important pathways and their components as well as the characterization of the predictive value of specific molecular changes have led to an improvement in individualization of the treatment of cancer patients. With increasing knowledge of molecular targets and biomarkers, it may become necessary to perform genomic profiling of a large number of genomic changes in cancer tissue, metastatic sites, and CTC prior to the implementation of anticancer therapies. Once multi-targeted drugs or combinations of targeted therapies become clinically applicable, genomic profiling may help to optimize treatment. Such profiling may require next-generation sequencing of a larger number and combination of genes, or profiling of many translational products. Currently, the technologies are limited. For example, the number of markers that can be analyzed is limited, regardless of their structures (i.e., proteins, mRNA, DNA, etc.). Whereas global profiling may add substantial information to the understanding of metastasis, heterogeneity of tumors, and the biology of disease, the current analyses are largely experimental. Analyses of specific changes, for now, may have a more specific clinical role in determining treatment strategies. The development of technologies capable of determining a large number of clinically relevant biomarkers is exigent.

Although in recent years tumor-specific genomic changes like copy number alterations or mutations have mostly been analyzed on cell free plasma DNA, many efforts have been

undertaken to profile CTC. Information expected from both sources may be different, since the information generated from CTC is more targeted. However, technical limitations of CTC isolation efficiency and the difficulties of performing whole-genome analyses on rare cells have limited the number of CTC genomic profile studies. In an early study, Paris et al. demonstrated that copy number profiles of CTC can distinguish them from remaining depleted mononuclear blood cells after EpCAM enrichment of CTC. CTC profiles detected in castration resistant prostate cancer patients were similar to their paired solid tumor DNA[33]. Magbanua et al.[34] delineated genomic alterations specific to CTC by comparative analysis between the CTC CGH dataset and a previously published dataset of primary tumor CGH[35] and focused on an important therapeutic target, focusing on potential gaing of HER2 in CTCs.

Mutation Analyses of CTC

Mutation analysis of genetic alterations predictive of response to targeted therapies in metastatic-stage cancer patients is currently performed on primary tumors. It would be appealing, however, to analyse actual disease, and CTC may provide one source for such analyses. Examples of mutations associated with therapy selection are *RAS* (KRAS and NRAS) or *BRAF* mutations in colocrectal cancer or *EGFR* in lung adenocarcinoma, respectively[36,37]. Punnoose et al have demonstrated the ability to detect KRAS mutations in captured CTC to predict response to targeted therapies[38]. Maheswaran et al. successfully detected *EGFR* mutations in CTC of metastatic lung cancer patients[11]. Yang et al.[39] and Mostert et al.[40] were also able to detect *KRAS* mutations in CTC in the peripheral blood of colorectal cancer patients. Androgen receptor mutation is a mechanisms leading to castration resistance in advanced prostate cancer[41]. Jiang et al.[42] have established an approach to detect such mutations in CTC prostate cancer patients. Technological advances in CTC enrichment and also in targeted sequencing have made it possible to perform genomic profiling also on CTC.

CTC Profiling beyond mutations

A variety of studies have now shown that CTC profiling can be performed on transcriptional level, but even greater interest focused on epigenomic and miRNA profiling. Smirnov et al. were one of the first to attempt global gene expression profiling of CTC in colorectal, prostate, and breast cancer patients[43]. The authors were able to differentiate the expression level of a set of CTC specific genes in patients compared to normal controls.

Since epigenetic events are fundamental to regulate development and differentiation, assessment of epigenomic alterations in CTC is regarded as crucial to understand the biology of cancer metastasis. So far, only few studies have evaluated DNA methylation in CTC, attempting to correlate CTC occurrence with the methylation status of circulating DNA[44–46]. We have recently shown, that we were able to successfully analyze CTC for a selection of methylated markers, and identified candidates for prognostic selection of breast cancer patients[47].

Cancer Stem Cells (CSC)

Experimental evidence in support of a “cancer stem cell model” in various malignancies is mounting[48]. The CSC model assumes the presence of a small proportion of cancer cells on top of a hierarchy of tumor cells; these CSC exhibit the capability to sustain tumor formation and growth, self-renewal and differentiation. Several markers of CSC have been identified, including a $CD44^{+}CD24^{-/low}$ phenotype and ALDH expression in breast and other cancers[49,50], and CD133 expression in non-small-cell lung cancer and brain tumors[51,52].

In breast cancer, and presumably other cancers, as well, the identification of distinct properties and molecular biomarkers of CSC may help in the development of more effective treatment and novel therapeutic targets[53]. In general, the presence of occult micrometastases is the rationale behind the use of systemic adjuvant chemotherapy following a definitive local treatment of a primary tumor[54]. The occult dissemination of CSC in bone marrow may be responsible for the failure of adjuvant chemotherapies in a proportion of early-stage breast cancer patients[55]. Several in vitro studies have demonstrated that putative breast CSC are resistant to conventional treatment strategies, including radiation and chemotherapy[56–58]. Consequently, the identification of breast CSC among CTC may be a promising strategy to assess their malignant potential and identify novel therapeutic targets. A major hurdle for such an approach is the hereto limited available knowledge regarding CTC phenotypes and the fact that CSC represent only a proportion of enriched CTC.

CSC in Dissemination

Pooled analysis of data from nine prior breast cancer studies which included a large number of early-stage patients revealed that the presence of disseminated tumor cells (DTC) in bone marrow is associated with a poor prognosis. Surprisingly, a significant proportion of patients with DTC had a favorable survival outcome 10 years or more following the diagnosis[59]. One potential reason for such an outcome could be the ability of DTC to remain dormant in distant organs. Based on the prior observations, we hypothesized that CSC not only exist within the primary tumor but may represent the most potent and virulent cells metastasizing from primary breast cancer to distant locations. In order to test our hypothesis, we performed a study analyzing DTC from breast cancer patients enrolled in the ACOS-OG Z-00010 trial for the putative breast CSC phenotype $CD44^{+}CD24^{-/low}$. The large majority of DTC in examined patients had the putative CSC phenotype[55]. This study provided the first demonstration that DTC/CTC are primarily composed of CSC, in contrast to primary and metastatic tumors in which fewer than 10% of cells have a CSC phenotype[55,60]. This finding has significant biologic implications, as it suggests that there is an enrichment for breast CSCs in the process of metastasis[61]. The fact that the ACOS-OG Z-00010 trial patients were early-stage I and II breast cancer patients in whom only 3% of BM samples were positive for DTC, made the finding even more significant[62].

Several studies have since confirmed these findings. In a prospective analysis of bone marrow aspirates from high-risk breast cancer patients, using cell sorting by flow cytometry, Reuben et al.[44] were able to show a high percentage of CSC in DTC. Using a similar approach, Theodoropoulos et al. demonstrated the presence of CTC in 67% of patients with

metastatic disease, with 35% of CTC displaying the $CD44^{+}/CD24^{low}$ CSC phenotype[45]. In another flow cytometry study, evaluating peripheral blood from breast cancer patients at variable stages, Wang et al. showed an increasing percentage of putative CSC in correlation with higher tumor stage[46]. The above findings further emphasize the need for reliable CTC enrichment methods allowing for detailed molecular characterization.

Epithelial–Mesenchymal Transition (EMT)

Progression to an EMT phenotype is widely accepted as a contributing factor to tumor metastasis and the ability of tumor cells to undergo EMT is crucial for local invasion and gaining access to the blood stream through intravasation[63]. EMT is associated with a specific set of genetic changes that lead to increased tumor cell motility and an invasive phenotype. These changes are typically characterized by loss of E-Cadherin expression and subsequent translocalization of β-catenin from the cell membrane into the nucleus, increased expression of vimentin, production of matrix metalloproteinase enzymes, and upregulation of various EMT-inducing transcription factors such as Twist, Snail, and Slug[64]. Thus, EMT provides a potential mechanistic basis for how CTC intravasate in primary tumors to reach the circulation, and subsequently extravasate from the circulation to seed tumor implants at distant secondary sites. Several studies have evaluated the expression of EMT associated markers in CTC. In a study involving metastatic breast cancer patients, Aktas et al. revealed at least one of three EMT markers (Akt2, PI3K, and Twist1), assessed by RT-PCR, to be expressed by the CTC population in 62% of patients harboring CTC. Patients with CTC who were positive for EMT were more likely to fail to respond to palliative chemotherapy, antibody or hormonal therapy[65]. Evaluating CTC expression of EMT markers Twist and vimentin by immunofluorescence, Kallergi et al. found vimentin/Twist expressing CTC in 77% of early-stage breast cancer patients compared to 100% of patients with metastatic disease[66]. In a recent study involving 11 breast cancer patients who were serially monitored for CTC phenotype, mesenchymal phenotype CTC were more likely to be associated with disease progression. In one index patient, the authors were able to demonstrate a reversible shift between the epithelial and mesenchymal phenotype corresponding to response to treatment and disease progression, respectively[67].

Recently, there has been described overlap between the EMT phenotype and the CSC phenotype. Overexpression of EMT transcription factors increases CSCs[68–70], such that EMT may thus drive both tumor dissemination and increase CSC self-renewal to facilitate tumor metastasis. Alternatively or additionally, the plasticity of tumor cells and their capability to transform and acquire mesenchymal characteristics may be derived from CSC[71]. To further complicate the link between EMT and the CSC phenotypes, we recently found that the CSC compartment can be epithelial and distinct from the mesenchymal compartment in a primary lung cancer cell culture we developed from a patient with primary resistant disease[72].

The CTC microenvironment

Recent studies show CTCs are not the only tumor elements that reach the peripheral blood. An accumulating body of evidence has demonstrated the pivotal role of stromal cells in

promoting cancer progression[73–76], metastasis and poor clinical outcome[77]. Cancer Associated Fibroblasts (CAFs), an essential component of the tumor microenvironment (TME)[78] in many cancers, comprise the majority of BC stromal cells. Stromal cells such as CAFs are not only present in the TME of the primary tumor, but exist in pre-metastatic and metastatic niches, and mouse metastasis models demonstrate that cancer cells take their CAFs with them to metastatic sites, and the survival and establishment of these CAFs promotes metastatic seeding and growth of the cancer cells[79]. Since successful metastatic seeding by CTCs is dependent on a productive interactive relationship with their environment as well as avoidance of immune surveillance, elucidation of interactions between CTCs and their microenvironment is critical. Such studies have been hindered primarily due to lack of a suitable platform to interrogate multiple cell types simultaneously; our unique microfilter platform enables such multicellular analyses as well as the capture and release of live circulating cells from patient blood. Using this platform, we discovered that non-cancer, non-immune cells were being isolated in association with CTCs and identified these as circulating CAFs (cCAFs)[80] (Figure 1J); we have also detected other cells of the tumor microenvironment in circulation, such as MDSC (Figure 1L). In a pilot study in which cCAFs and CTCs were enumerated in the blood of breast cancer patients, we found cCAFs present in patients with Stage IV (metastatic) breast cancer but not in blood from patients with likely cured Stage I disease, while CTCs were detected in both[80]. Jones et al. also found circulating fibroblast-like cells, identified as CK-/CD45-/vimentin+, in the blood of metastatic prostate cancer patients[81]. More recently, we have used this platform to capture and enumerate CTCs and cCAFs from mouse blood in mouse xenograft models (Figure 1K).

In addition to CAFs, tumor associated macrophages (TAMs) have also been identified in circulation[82]. Circulating immune cells such as cytotoxic T cells (T_{cyt}) play important roles in tumor suppression and other immune cells such as myelo-derived suppressor cells (MDSCs), regulatory T cells (T_{regs}), and TAMs play important roles in tumor enhancement (reviewed in[83]). MDSCs are recruited to tumors and metastases and actively suppress T_{cyt} and this suppression of immune response results in enhanced tumor growth and metastasis (reviewed in[84]). Depletion of MDSCs in mouse breast cancer models results in reduced tumor growth and inhibition of metastasis[85]. Using the MDSC marker S100A8[85], we have been able to demonstrate the presence of S100A8+ cells in blood from mice with metastatic syngeneic breast tumors with our platform.

CTC Clusters

Additional potential mechanisms that could facilitate tumor cell dissemination include ameboid motility and collective migration of cell clusters[86]. In mouse breast cancer models, CTC clusters exhibit higher metastatic capacity compared with individual or single CTCs[87]. Additionally, it was demonstrated that polyclonal breast cancer metastasis resulted from CTC clusters composed of Keratin 14+ CTCs[88]. Recently, CTC cluster enumeration in breast cancer patients have demonstrated that like CTC number, the presence of CTC clusters correlates with reduced progression-free survival and poor outcome[88–91]. Recent data suggests that CTC clusters that are composed of CSCs, thus CSC clusters, are more metastatic than CTC clusters composed of none-CSC CTCs; these studies have also demonstrated the presence of CSC clusters in patient blood[92]. Interestingly, in these studies

the CSCs exhibited a hybrid epithelial/mesenchymal phenotype. The occurrence of circulating tumor micro-emboli (CTM) in metastatic lung cancer patients was demonstrated by Hou et al.[93]. In this study, the authors showed that single CTC expressed apoptosis related markers at a higher rate than CTM. These findings suggest that collective migration of tumor cells in circulation may offer a survival advantage to the tumor.

We demonstrated that cCAFs can cluster with CTCs as well as with each other. In mouse models of both human breast cancer xenografts as well as genetically engineered spontaneous breast cancer, both individual CTCs and cCAFs as well as abundant cCAF/CTC clusters can be captured and enumerated from 100 ul of mouse blood using our microfilter platform. We have established in vitro cluster assays and can also capture these clusters using our platform. These mouse blood and in vitro cluster assay CTC and cCAF capture were all performed using the automated version of our platform, FaCTChecker.

Thus, it may be that it not solely CTC clusters that are responsible for metastatic seeding, but rather it is clusters comprised of CTCs along with their tumor microenvironment, including cCAFs that are the metastasis-driving factors. Given the recent evidence that at least a portion of CTCs are cells transitioning between the epithelial and mesenchymal state[67] that possess stem cell-like properties and the ability of reversible modulation[94], the functional characterization of these processes in CTCs, and in these CTC/stromal cell clusters is crucial. Development of new technologies that will enhance sensitivity and efficiency of CTC and CTC cluster detection will facilitate functional characterization of CTC invasiveness, aggressiveness, plasticity, and tumorigenic potential. Functional characterization will, in turn, help further clarify the mechanisms of tumor cell dissemination.

CTC Propagation

Although many CTC are shed from the primary tumor, only a few will survive and successfully colonize in distant organs, and develop into distal metastasis. The ability to expand these elusive cells in vitro would create a new class of biospecimen, available for detailed precision analyses. It would deliver a major new tool that supports hypothesis driven questions related to the cascade of events leading to metastasis, serve as a high throughput platform for screening novel therapies that may arrest this process, and a critical advance in precision cancer management. CTC have notoriously heterogeneous inherent proliferative capacity, as revealed by variations in Ki67 expression (antigen expressed in proliferating cells but absent in quiescent cells)[95,96], and stem-cell markers[97–100]. It is imperative that the methods to recover the captured CTC are agnostic to presence of specific molecular targets, so that CTC heterogeneity is not artificially diminished. Building upon our very successful size-based strategy for CTCs[28], and its variation employing slot-shaped pores for viable CTC capture and long term maintenance,[27,101–103], we reported capture and thermoresponsive release of viable cancer cells from our microfilter surface[104]. Poly(N-iso-propylacrylamide) (PIPAAm) is a polymer that undergoes a reversible lower critical solution temperature (LCST) phase transition at a solution temperature of 32 °C. In our formulation, CTC capture is performed at room temperature (below 32 °C) on PIPAAm coated microfilters, and cell release is enabled by placing the filter in culture media maintained at

37 °C (Figure 1C). At this temperature, the PIPAAm polymer layer becomes hydrophobic, thereby releasing the electrostatically bound cells. After demonstrating that the release was feasible with cultured cancer cells, we continued to evaluate the impact of thermal release on conditionally reprogrammed cells established from a non-small cell lung cancer patient. Even when spiked to blood, these cells could be captured using our slot filter, thermically released, and successfully cultured again. Capture and subsequent release had no impact on the viability and functionality of cells, thus demonstrating the procedure is applicable to human cancer cells[104].

With these advances in place, we are evaluating tissue engineered microenvironments that recapitulate the metastatic niche (such as bone), and might be instructive in maintaining CTC cultures, and even propagating them. CTC from various malignancies are attracted to bone marrow given the fertile environment it provides to establish secondary metastatic tumors. The process of metastasis to bone requires a close interaction of invading CTC with local stromal cells of mesenchymal phenotype, situated at the perivascular niche, and the parenchyma, as well as with MSC-derived secretory osteoblasts[105]. The interaction and resulting intercellular cross-talk has been implicated in determining the fate of the metastatic tumors. Therefore, *in vitro* recapitulation of the target tissue microenvironment could provide “natural” habitats for CTC to thrive.

In the last three years, a few reports have been published on propagating CTC with the goal of creating CTC cultures from breast, colon, lung and prostate cancers[87,106–111]. All studies that attempt to propagate CTC share the following features: (a) requirement of high starting numbers of CTC in the blood (3-3000 cells/10-20ml blood), (b) low culture efficiency, (c) use of non- or low adherent culturing conditions, and (d) development of initial cultures in small volumes. The reported successful cultures include development of 1 colon cancer CTC cell line (out of 30 patients)[106], 1 prostate cancer cell line (out of 17 patients)[108], and 6 breast cancer cell lines (out of 36 patients)[107,110]. Recent studies have reported greater success in developing short-term cultures using smaller starting numbers of CTC co-cultured with fibroblasts[109], CTC clusters[87,111], and successful xenograft propagation of lung cancer CTC[112]. While limited, the success of these approaches strongly indicates that the microenvironment may be critical to successful CTC culture[113].

Conclusion

CTC provide unique opportunities for real-time monitoring of disease progression and treatment response. Development of increasingly more sensitive technologies, particularly EpCAM-independent approaches, as well as techniques for robust molecular and functional characterization of these cells will offer clues to the mechanisms of how cancer develop resistance to therapies, and how they spread to distant organs. In parallel, development of integrated culture and interrogation platform for CTCs will be an exceptionally powerful oncology toolset for discovery of new therapeutics, and precision cancer management.

References

1. Alix-Panabieres C, Pantel K. Circulating tumor cells: liquid biopsy of cancer. Clin Chem. 59(1): 110–118.

2. Balic M, Williams A, Dandachi N, Cote RJ. Micrometastasis: detection methods and clinical importance. Cancer Biomark. 2010; 9(1-6):397–419. [PubMed: 22112487]

3. Lin H, Balic M, Zheng S, Datar R, Cote RJ. Disseminated and circulating tumor cells: Role in effective cancer management. Crit Rev Oncol Hematol. 2011; 77(1):1–11. [PubMed: 20570170]

4. Rawal S, Yang YP, Cote R, Agarwal A. Identification and Quantitation of Circulating Tumor Cells. Annu Rev Anal Chem (Palo Alto Calif). 2017; 10(1):321–343. [PubMed: 28301753]

5. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med. 2004; 351(8):781–791. [PubMed: 15317891]

6. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, et al. Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature. 2007; 450(7173):1235–1239. [PubMed: 18097410]

7. Liu Z, Fusi A, Klopocki E, Schmittel A, Tinhofer I, Nonnenmacher A, et al. Negative enrichment by immunomagnetic nanobeads for unbiased characterization of circulating tumor cells from peripheral blood of cancer patients. J Transl Med. 2011; 9:70. [PubMed: 21595914]

8. Lustberg M, Jatana KR, Zborowski M, Chalmers JJ. Emerging technologies for CTC detection based on depletion of normal cells. Recent Results Cancer Res. 2012; 195:97–110. [PubMed: 22527498]

9. Naume B, Borgen E, Beiske K, Herstad TK, Ravnas G, Renolen A, et al. Immunomagnetic techniques for the enrichment and detection of isolated breast carcinoma cells in bone marrow and peripheral blood. J Hematother. 1997; 6(2):103–114. [PubMed: 9131439]

10. Iinuma H, Okinaga K, Adachi M, Suda K, Sekine T, Sakagawa K, et al. Detection of tumor cells in blood using CD45 magnetic cell separation followed by nested mutant allele-specific amplification of p53 and K-ras genes in patients with colorectal cancer. Int J Cancer. 2000; 89(4):337–344. [PubMed: 10956407]

11. Maheswaran S, Sequist LV, Nagrath S, Ulkus L, Brannigan B, Collura CV, et al. Detection of mutations in EGFR in circulating lung-cancer cells. N Engl J Med. 2008; 359(4):366–377. [PubMed: 18596266]

12. de Wit S, van Dalum G, Lenferink AT, Tibbe AG, Hiltermann TJ, Groen HJ, et al. The detection of EpCAM(+) and EpCAM(-) circulating tumor cells. Sci Rep. 2015; 5:12270. [PubMed: 26184843]

13. Gorges TM, Tinhofer I, Drosch M, Rose L, Zollner TM, Krahn T, et al. Circulating tumour cells escape from EpCAM-based detection due to epithelial-to-mesenchymal transition. BMC Cancer. 2012; 12:178. [PubMed: 22591372]

14. Schneck H, Gierke B, Uppenkamp F, Behrens B, Niederacher D, Stoecklein NH, et al. EpCAM-Independent Enrichment of Circulating Tumor Cells in Metastatic Breast Cancer. PLoS One. 2015; 10(12):e0144535. [PubMed: 26695635]

15. Galletti G, Sung MS, Vahdat LT, Shah MA, Santana SM, Altavilla G, et al. Isolation of breast cancer and gastric cancer circulating tumor cells by use of an anti HER2-based microfluidic device. Lab Chip. 2014; 14(1):147–156. [PubMed: 24202699]

16. Kirby BJ, Jodari M, Loftus MS, Gakhar G, Pratt ED, Chanel-Vos C, et al. Functional characterization of circulating tumor cells with a prostate-cancer-specific microfluidic device. PLoS One. 2012; 7(4):e35976. [PubMed: 22558290]

17. Winer-Jones JP, Vahidi B, Arquilevich N, Fang C, Ferguson S, Harkins D, et al. Circulating tumor cells: clinically relevant molecular access based on a novel CTC flow cell. PLoS One. 2014; 9(1):e86717. [PubMed: 24489774]

18. Satelli A, Brownlee Z, Mitra A, Meng QH, Li S. Circulating tumor cell enumeration with a combination of epithelial cell adhesion molecule- and cell-surface vimentin-based methods for monitoring breast cancer therapeutic response. Clin Chem. 2015; 61(1):259–266. [PubMed: 25336717]

19. Grover PK, Cummins AG, Price TJ, Roberts-Thomson IC, Hardingham JE. Circulating tumour cells: the evolving concept and the inadequacy of their enrichment by EpCAM-based methodology for basic and clinical cancer research. Ann Oncol. 2014; 25(8):1506–1516. [PubMed: 24651410]

20. Kowalik A, Kowalewska M, Gozdz S. Current approaches for avoiding the limitations of circulating tumor cells detection methods-implications for diagnosis and treatment of patients with solid tumors. Transl Res. 2017; 185:58–84 e15. [PubMed: 28506696]
21. de Wit S, van Dalum G, Terstappen LW. Detection of circulating tumor cells. Scientifica (Cairo). 2014; 2014:819362. [PubMed: 25133014]
22. Karabacak NM, Spuhler PS, Fachin F, Lim EJ, Pai V, Ozkumur E, et al. Microfluidic, marker-free isolation of circulating tumor cells from blood samples. Nat Protoc. 2014; 9(3):694–710. [PubMed: 24577360]
23. Ferreira MM, Ramani VC, Jeffrey SS. Circulating tumor cell technologies. Mol Oncol. 2016; 10(3):374–394. [PubMed: 26897752]
24. Rosenberg R, Gertler R, Friederichs J, Fuehrer K, Dahm M, Phelps R, et al. Comparison of two density gradient centrifugation systems for the enrichment of disseminated tumor cells in blood. Cytometry. 2002; 49(4):150–158. [PubMed: 12454978]
25. Fleischer RL. Cancer filter deja vu. Science. 2007; 318(5858):1864.
26. Vona G, Sabile A, Louha M, Sitruk V, Romana S, Schutze K, et al. Isolation by size of epithelial tumor cells : a new method for the immunomorphological and molecular characterization of circulatingtumor cells. Am J Pathol. 2000; 156(1):57–63. [PubMed: 10623654]
27. Lin HK, Zheng S, Williams AJ, Balic M, Groshen S, Scher HI, et al. Portable filter-based microdevice for detection and characterization of circulating tumor cells. Clin Cancer Res. 2010; 16(20):5011–5018. [PubMed: 20876796]
28. Zheng S, Lin H, Liu JQ, Balic M, Datar R, Cote RJ, et al. Membrane microfilter device for selective capture, electrolysis and genomic analysis of human circulating tumor cells. J Chromatogr A. 2007; 1162(2):154–161. [PubMed: 17561026]
29. Cote RJ, Beattie EJ, Chaiwun B, Shi SR, Harvey J, Chen SC, et al. Detection of occult bone marrow micrometastases in patients with operable lung carcinoma. Ann Surg. 1995; 222(4):415–423. discussion 423-415. [PubMed: 7574923]
30. White JG, Amos WB, Fordham M. An evaluation of confocal versus conventional imaging of biological structures by fluorescence light microscopy. The Journal of Cell Biology. 1987; 105(1): 41–48. [PubMed: 3112165]
31. Williams A, Chung J, Ou X, Zheng G, Rawal S, Ao Z, et al. Fourier ptychographic microscopy for filtration-based circulating tumor cell enumeration and analysis. Journal of biomedical optics. 2014; 19(6):066007. [PubMed: 24949708]
32. Zheng GA, Horstmeyer R, Yang CH. Wide-field, high-resolution Fourier ptychographic microscopy. Nat Photonics. 2013; 7(9):739–745. [PubMed: 25243016]
33. Paris PL, Kobayashi Y, Zhao Q, Zeng W, Sridharan S, Fan T, et al. Functional phenotyping and genotyping of circulating tumor cells from patients with castration resistant prostate cancer. Cancer Lett. 2009; 277(2):164–173. [PubMed: 19162393]
34. Magbanua MJ, Sosa EV, Roy R, Eisenbud LE, Scott JH, Olshen A, et al. Genomic profiling of isolated circulating tumor cells from metastatic breast cancer patients. Cancer Res. 73(1):30–40.
35. Fridlyand J, Snijders AM, Ylstra B, Li H, Olshen A, Segraves R, et al. Breast tumor copy number aberration phenotypes and genomic instability. BMC Cancer. 2006; 6:96. [PubMed: 16620391]
36. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008; 26(10):1626–1634. [PubMed: 18316791]
37. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science. 2004; 304(5676):1497–1500. [PubMed: 15118125]
38. Punnoose EA, Atwal SK, Spoerke JM, Savage H, Pandita A, Yeh RF, et al. Molecular biomarker analyses using circulating tumor cells. PLoS One. 5(9):e12517.
39. Yang MJ, Chiu HH, Wang HM, Yen LC, Tsao DA, Hsiao CP, et al. Enhancing detection of circulating tumor cells with activating KRAS oncogene in patients with colorectal cancer by weighted chemiluminescent membrane array method. Ann Surg Oncol. 17(2):624–633.

40. Mostert B, Jiang Y, Sieuwerts AM, Wang H, Bolt-de Vries J, Biermann K, et al. KRAS and BRAF mutation status in circulating colorectal tumor cells and their correlation with primary and metastatic tumor tissue. Int J Cancer.
41. Taplin ME, Rajeshkumar B, Halabi S, Werner CP, Woda BA, Picus J, et al. Androgen receptor mutations in androgen-independent prostate cancer: Cancer and Leukemia Group B Study 9663. J Clin Oncol. 2003; 21(14):2673–2678. [PubMed: 12860943]
42. Jiang Y, Palma JF, Agus DB, Wang Y, Gross ME. Detection of androgen receptor mutations in circulating tumor cells in castration-resistant prostate cancer. Clin Chem. 56(9):1492–1495.
43. Smirnov DA, Zweitzig DR, Foulk BW, Miller MC, Doyle GV, Pienta KJ, et al. Global gene expression profiling of circulating tumor cells. Cancer Res. 2005; 65(12):4993–4997. [PubMed: 15958538]
44. Reuben JM, Lee BN, Gao H, Cohen EN, Mego M, Giordano A, et al. Primary breast cancer patients with high risk clinicopathologic features have high percentages of bone marrow epithelial cells with ALDH activity and CD44CD24lo cancer stem cell phenotype. Eur J Cancer. 2011; 47(10):1527–1536. [PubMed: 21334874]
45. Theodoropoulos PA, Polioudaki H, Agelaki S, Kallergi G, Saridaki Z, Mavroudis D, et al. Circulating tumor cells with a putative stem cell phenotype in peripheral blood of patients with breast cancer. Cancer Lett. 288(1):99–106.
46. Wang N, Shi L, Li H, Hu Y, Du W, Liu W, et al. Detection of circulating tumor cells and tumor stem cells in patients with breast cancer by using flow cytometry : A valuable tool for diagnosis and prognosis evaluation. Tumour Biol. 2012; 33(2):561–569. [PubMed: 22241087]
47. Benezeder T, Tiran V, Treitler AAN, Suppan C, Rossmann C, Stoeger H, et al. Multigene methylation analysis of enriched circulating tumor cells associates with poor progression-free survival in metastatic breast cancer patients. Oncotarget. 2017; 8(54):92483–92496. [PubMed: 29190932]
48. Azizi E, Wicha MS. Cancer Stem Cells–The Evidence Accumulates. Clin Chem. 2012
49. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell. 2007; 1(5):555–567. [PubMed: 18371393]
50. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A. 2003; 100(7):3983–3988. [PubMed: 12629218]
51. Wang S, Xu ZY, Wang LF, Su W. CD133+ cancer stem cells in lung cancer. Front Biosci;. 18:447–453.
52. Singh SK, Clarke ID, Hide T, Dirks PB. Cancer stem cells in nervous system tumors. Oncogene. 2004; 23(43):7267–7273. [PubMed: 15378086]
53. Schardt JA, Meyer M, Hartmann CH, Schubert F, Schmidt-Kittler O, Fuhrmann C, et al. Genomic analysis of single cytokeratin-positive cells from bone marrow reveals early mutational events in breast cancer. Cancer Cell. 2005; 8(3):227–239. [PubMed: 16169467]
54. Cote RJ. Occult metastases: real harm or false alarm? J Thorac Cardiovasc Surg. 2003; 126(2): 332–333. [PubMed: 12928627]
55. Balic M, Lin H, Young L, Hawes D, Giuliano A, McNamara G, et al. Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. Clin Cancer Res. 2006; 12(19):5615–5621. [PubMed: 17020963]
56. Duru N, Fan M, Candas D, Menaa C, Liu HC, Nantajit D, et al. HER2-associated radiation resistance of breast cancer stem cells isolated from HER2-negative breast cancer cells. Clin Cancer Res. 2012
57. Fillmore CM, Kuperwasser C. Human breast cancer cell lines contain stem-like cells that self-renew, give rise to phenotypically diverse progeny and survive chemotherapy. Breast Cancer Res. 2008; 10(2):R25. [PubMed: 18366788]
58. Kakarala M, Wicha MS. Implications of the cancer stem-cell hypothesis for breast cancer prevention and therapy. J Clin Oncol. 2008; 26(17):2813–2820. [PubMed: 18539959]

59. Braun S, Vogl FD, Naume B, Janni W, Osborne MP, Coombes RC, et al. A pooled analysis of bone marrow micrometastasis in breast cancer. N Engl J Med. 2005; 353(8):793–802. [PubMed: 16120859]
60. Abraham BK, Fritz P, McClellan M, Hauptvogel P, Athelogou M, Brauch H. Prevalence of CD44+/CD24-/low cells in breast cancer may not be associated with clinical outcome but may favor distant metastasis. Clin Cancer Res. 2005; 11(3):1154–1159. [PubMed: 15709183]
61. Wicha MS. Cancer stem cells and metastasis: lethal seeds. Clin Cancer Res. 2006; 12(19):5606–5607. [PubMed: 17020960]
62. Giuliano AE, Hawes D, Ballman KV, Whitworth PW, Blumencranz PW, Reintgen DS, et al. Association of occult metastases in sentinel lymph nodes and bone marrow with survival among women with early-stage invasive breast cancer. JAMA. 2011; 306(4):385–393. [PubMed: 21791687]
63. Lim J, Thiery JP. Epithelial-mesenchymal transitions: insights from development. Development. 2012; 139(19):3471–3486. [PubMed: 22949611]
64. Lee K, Nelson CM. New insights into the regulation of epithelial-mesenchymal transition and tissue fibrosis. Int Rev Cell Mol Biol. 294:171–221.
65. Aktas B, Tewes M, Fehm T, Hauch S, Kimmig R, Kasimir-Bauer S. Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. Breast Cancer Res. 2009; 11(4):R46. [PubMed: 19589136]
66. Ignatiadis M, Kallergi G, Ntoulia M, Perraki M, Apostolaki S, Kafousi M, et al. Prognostic value of the molecular detection of circulating tumor cells using a multimarker reverse transcription-PCR assay for cytokeratin 19, mammaglobin A, and HER2 in early breast cancer. Clin Cancer Res. 2008; 14(9):2593–2600. [PubMed: 18451221]
67. Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, Ting DT, et al. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science. 339(6119):580–584.
68. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. 2008; 133(4):704–715. [PubMed: 18485877]
69. Morel AP, Lievre M, Thomas C, Hinkal G, Ansieau S, Puisieux A. Generation of breast cancer stem cells through epithelial-mesenchymal transition. PLoS One. 2008; 3(8):e2888. [PubMed: 18682804]
70. Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, et al. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nat Cell Biol. 2009; 11(12):1487–1495. [PubMed: 19935649]
71. Scheel C, Weinberg RA. Phenotypic plasticity and epithelial-mesenchymal transitions in cancer and normal stem cells? Int J Cancer. 2011; 129(10):2310–2314. [PubMed: 21792896]
72. Tiran V, Lindenmann J, Brcic L, Heitzer E, Stanzer S, Tabrizi-Wizsy NG, et al. Primary patient-derived lung adenocarcinoma cell culture challenges the association of cancer stem cells with epithelial-to-mesenchymal transition. Sci Rep. 2017; 7(1):10040. [PubMed: 28855609]
73. Tlsty TD. Stromal cells can contribute oncogenic signals. Semin Cancer Biol. 2001; 11(2):97–104. [PubMed: 11322829]
74. Allinen M, Beroukhim R, Cai L, Brennan C, Lahti-Domenici J, Huang H, et al. Molecular characterization of the tumor microenvironment in breast cancer. Cancer cell. 2004; 6(1):17–32. [PubMed: 15261139]
75. Nguyen DX, Bos PD, Massague J. Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer. 2009; 9(4):274–U265. [PubMed: 19308067]
76. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144(5):646–674. [PubMed: 21376230]
77. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer. 2006; 6(5):392–401. [PubMed: 16572188]
78. Orimo A, Weinberg RA. Stromal fibroblasts in cancer: a novel tumor-promoting cell type. Cell cycle. 2006; 5(15):1597–1601. [PubMed: 16880743]

79. Duda DG, Duyverman AM, Kohno M, Snuderl M, Steller EJ, Fukumura D, et al. Malignant cells facilitate lung metastasis by bringing their own soil. Proc Natl Acad Sci U S A. 2010; 107(50): 21677–21682. [PubMed: 21098274]
80. Ao Z, Shah SH, Machlin LM, Parajuli R, Miller PC, Rawal S, et al. Identification of Cancer-Associated Fibroblasts in Circulating Blood from Patients with Metastatic Breast Cancer. Cancer research. 2015; 75(22):4681–4687. [PubMed: 26471358]
81. Jones ML, Siddiqui J, Pienta KJ, Getzenberg RH. Circulating fibroblast-like cells in men with metastatic prostate cancer. The Prostate. 2013; 73(2):176–181. [PubMed: 22718300]
82. Adams DL, Martin SS, Alpaugh RK, Charpentier M, Tsai S, Bergan RC, et al. Circulating giant macrophages as a potential biomarker of solid tumors. Proc Natl Acad Sci U S A. 2014; 111(9): 3514–3519. [PubMed: 24550495]
83. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013; 19(11):1423–1437. [PubMed: 24202395]
84. Nagaraj S, Gabrilovich DI. Myeloid-derived suppressor cells in human cancer. Cancer J. 2010; 16(4):348–353. [PubMed: 20693846]
85. Drews-Elger K, Iorns E, Dias A, Miller P, Ward TM, Dean S, et al. Infiltrating S100A8+ myeloid cells promote metastatic spread of human breast cancer and predict poor clinical outcome. Breast Cancer Res Treat. 2014; 148(1):41–59. [PubMed: 25270120]
86. Friedlein R, von Kieseritzky F, Braun S, Linde C, Osikowicz W, Hellberg J, et al. Solution-processed, highly-oriented supramolecular architectures of functionalized porphyrins with extended electronic states. Chem Commun (Camb). 2005; (15):1974–1976. [PubMed: 15834476]
87. Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell. 2014; 158(5):1110–1122. [PubMed: 25171411]
88. Cheung KJ, Padmanaban V, Silvestri V, Schipper K, Cohen JD, Fairchild AN, et al. Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters. Proc Natl Acad Sci U S A. 2016; 113(7):E854–863. [PubMed: 26831077]
89. Jansson S, Bendahl PO, Larsson AM, Aaltonen KE, Ryden L. Prognostic impact of circulating tumor cell apoptosis and clusters in serial blood samples from patients with metastatic breast cancer in a prospective observational cohort. BMC Cancer. 2016; 16:433. [PubMed: 27390845]
90. Mu Z, Benali-Furet N, Uzan G, Znaty A, Ye Z, Paolillo C, et al. Detection and Characterization of Circulating Tumor Associated Cells in Metastatic Breast Cancer. Int J Mol Sci. 2016; 17(10)
91. Wang C, Mu Z, Chervoneva I, Austin L, Ye Z, Rossi G, et al. Longitudinally collected CTCs and CTC-clusters and clinical outcomes of metastatic breast cancer. Breast Cancer Res Treat. 2017; 161(1):83–94. [PubMed: 27771841]
92. Jolly MK, Boareto M, Debeb BG, Aceto N, Farach-Carson MC, Woodward WA, et al. Inflammatory breast cancer: a model for investigating cluster-based dissemination. NPJ Breast Cancer. 2017; 3:21. [PubMed: 28649661]
93. Hou JM, Krebs M, Ward T, Sloane R, Priest L, Hughes A, et al. Circulating tumor cells as a window on metastasis biology in lung cancer. Am J Pathol. 178(3):989–996.
94. May CD, Sphyris N, Evans KW, Werden SJ, Guo W, Mani SA. Epithelial-mesenchymal transition and cancer stem cells: a dangerously dynamic duo in breast cancer progression. Breast Cancer Res. 13(1):202.
95. Kallergi G, Konstantinidis G, Markomanolaki H, Papadaki MA, Mavroudis D, Stournaras C, et al. Apoptotic Circulating Tumor Cells in Early and Metastatic Breast Cancer Patients. Mol Cancer Ther. 2013; 12(9):1886–1895. [PubMed: 23778153]
96. Muller V, Stahmann N, Riethdorf S, Rau T, Zabel T, Goetz A, et al. Circulating tumor cells in breast cancer: Correlation to bone marrow micrometastases, heterogeneous response to systemic therapy and low proliferative activity. Clin Cancer Res. 2005; 11(10):3678–3685. [PubMed: 15897564]
97. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. P Natl Acad Sci USA. 2003; 100(7):3983–3988.

98. Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, et al. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. Cancer Res. 2005; 65(13):5506–5511. [PubMed: 15994920]

99. Theodoropoulos PA, Polioudaki H, Agelaki S, Kallergi G, Saridaki Z, Mavroudis D, et al. Circulating tumor cells with a putative stem cell phenotype in peripheral blood of patients with breast cancer. Cancer Lett. 2010; 288(1):99–106. [PubMed: 19619935]

100. Kallergi G, Agelaki S, Kalykaki A, Stournaras C, Mavroudis D, Georgoulias V. Phosphorylated EGFR and PI3K/Akt signaling kinases are expressed in circulating tumor cells of breast cancer patients. Breast Cancer Res. 2008; 10(5):R80. [PubMed: 18822183]

101. Zhou MD, Hao S, Williams AJ, Harouaka RA, Schrand B, Rawal S, et al. Separable bilayer microfiltration device for viable label-free enrichment of circulating tumour cells. Sci Rep. 2014; 4:7392. [PubMed: 25487434]

102. Zheng S, Lin HK, Lu B, Williams A, Datar R, Cote RJ, et al. 3D microfilter device for viable circulating tumor cell (CTC) enrichment from blood. Biomed Microdevices. 2011; 13(1):203–213. [PubMed: 20978853]

103. Xu T, Lu B, Tai YC, Goldkorn A. A cancer detection platform which measures telomerase activity from live circulating tumor cells captured on a microfilter. Cancer Res. 2010; 70(16): 6420–6426. [PubMed: 20663903]

104. Ao Z, Parasido E, Rawal S, Williams A, Schlegel R, Liu S, et al. Thermoresponsive release of viable microfiltrated Circulating Tumor Cells (CTCs) for precision medicine applications. Lab Chip. 2015; 15(22):4277–4282. [PubMed: 26426331]

105. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. Nat Rev Cancer. 2009; 9(4): 239–252. [PubMed: 19279573]

106. Cayrefourcq L, Mazard T, Joosse S, Solassol J, Ramos J, Assenat E, et al. Establishment and characterization of a cell line from human circulating colon cancer cells. Cancer Res. 2015; 75(5):892–901. [PubMed: 25592149]

107. Yu M, Bardia A, Aceto N, Bersani F, Madden MW, Donaldson MC, et al. Cancer therapy. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. Science. 2014; 345(6193):216–220. [PubMed: 25013076]

108. Gao D, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, Gopalan A, et al. Organoid cultures derived from patients with advanced prostate cancer. Cell. 2014; 159(1):176–187. [PubMed: 25201530]

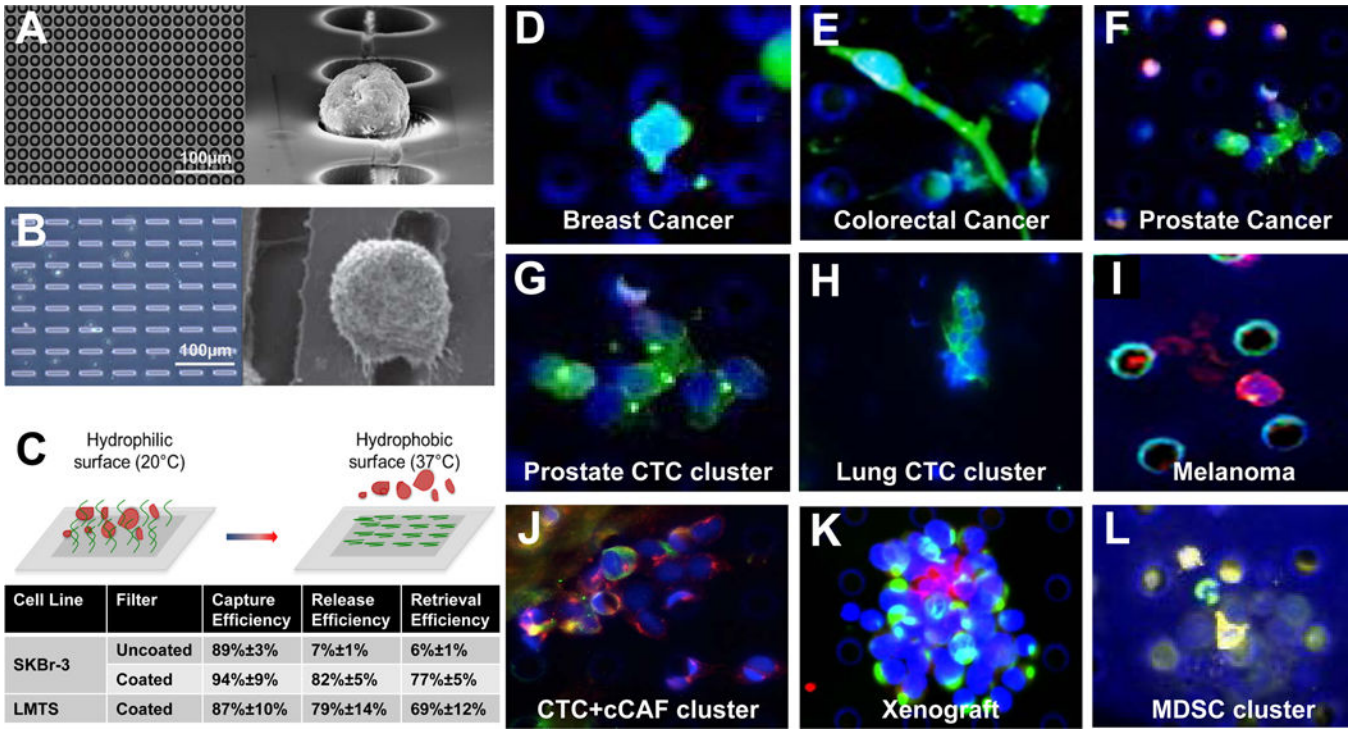
109. Zhang Z, Shiratsuchi H, Lin J, Chen G, Reddy RM, Azizi E, et al. Expansion of CTCs from early stage lung cancer patients using a microfluidic co-culture model. Oncotarget. 2014; 5(23):12383–12397. [PubMed: 25474037]

110. Jordan NV, Bardia A, Wittner BS, Benes C, Ligorio M, Zheng Y, et al. HER2 expression identifies dynamic functional states within circulating breast cancer cells. Nature. 2016; 537(7618):102–106. [PubMed: 27556950]

111. Cheung KJ, Ewald AJ. A collective route to metastasis: Seeding by tumor cell clusters. Science. 2016; 352(6282):167–169. [PubMed: 27124449]

112. Hodgkinson CL, Morrow CJ, Li Y, Metcalf RL, Rothwell DG, Trapani F, et al. Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer. Nat Med. 2014; 20(8): 897–903. [PubMed: 24880617]

113. Bichsel CA, Gobaa S, Kobel S, Secondini C, Thalmann GN, Cecchini MG, et al. Diagnostic microchip to assay 3D colony-growth potential of captured circulating tumor cells. Lab Chip. 2012; 12(13):2313–2316. [PubMed: 22565166]



Cell Line	Filter	Capture Efficiency	Release Efficiency	Retrieval Efficiency
SKBr-3	Uncoated	89%±3%	7%±1%	6%±1%
	Coated	94%±9%	82%±5%	77%±5%
LMTS	Coated	87%±10%	79%±14%	69%±12%

Figure 1. Membrane microfilter technology for CTC capture and release

(A) 8 μm diameter round pore microfilters are used to capture fixed cells for CTC enumeration and characterization. Also shown is a scanning electron micrograph of fixed tumor cells captured by the round pore microfilter. (B) Slot pore microfilters (6 μm × 40 μm) are used for viable CTC capture, with a Scanning electron micrographs of fixed tumor cells captured by slot pore microfilter. **(D-I): Microfilter technology is versatile for clinical samples from several different malignancies.** Single CTC from (D) Breast cancer, (E) Colorectal cancer, (F) Prostate cancer captured, immunostained, and enumerated as a part of Cleveland Clinic collaborative study. CTC clusters from (G) Prostate cancer and (H) Lung cancer patients captured on the microfilter. (I) Detection of non-epithelial CTC from Melanoma samples shows the important distinction of microfilter technology from other (mostly EpCAM-binding dependent) commercially available platforms. Green: cytokeratin; Blue: DAPI; (in I) Red: Anti-melanoma antibody cocktail. **(J-L): Microfilter device captures intact clusters of CTCs with cCAFs and other tumor microenvironment cells.** (J) CTC+cCAF cluster from human patient with metastatic breast cancer. (K) CTC+cCAF cluster from xenograft model of breast cancer metastasis. Green: cytokeratin; Red: FAP; Blue: DAPI. (L) S100A8-positive MDSC cells (Yellow) clustering with breast cancer cells from murine syngeneic metastatic breast cancer model (4T1 cells, Green).

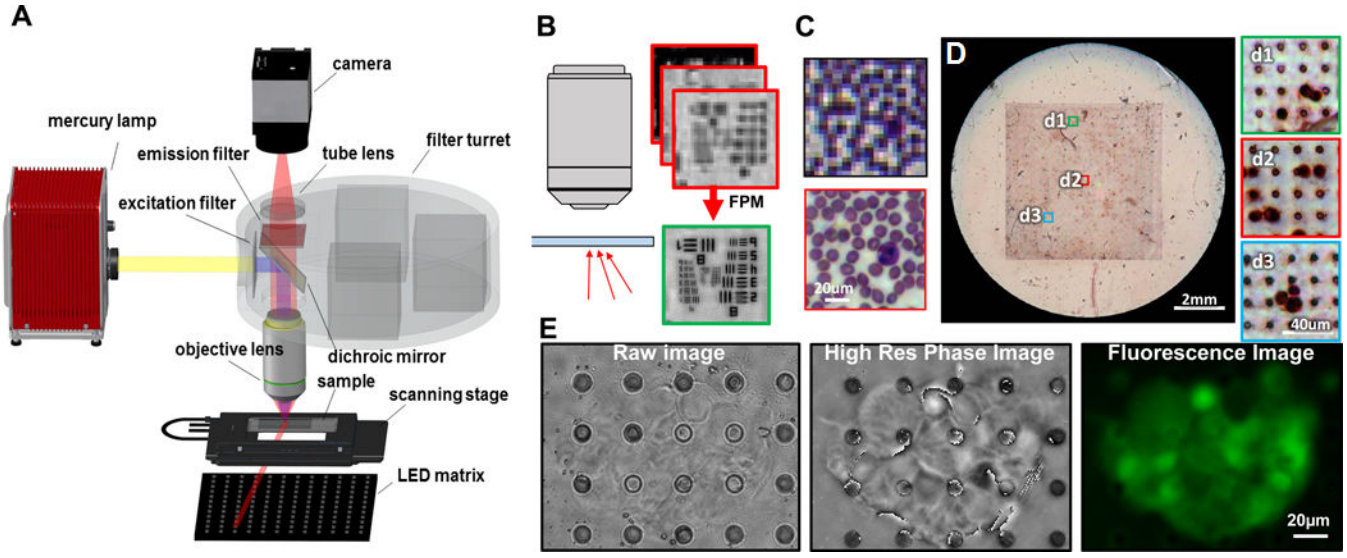


Figure 2. FPM applied to imaging CTC microfilter

(A) Schematic of FPM + fluorescence imaging system. FPM images are captured with the LED matrix illumination. Fluorescence images of multiple channels are captured by switching to different fluorescence filter cubes in the filter turret and using the epi-illumination provided by a mercury lamp. The slide is scanned laterally for a full field-of-view image. (B) Series of low resolution images of the slide is acquired with different illumination angle. The ptychographic algorithm then stitches the data together in spatial frequency space and titrate a final image that has both phase and amplitude information and improved resolution. (C) A typical FPM raw image and the corresponding FPM titrated image, showing a resolution improvement of 5 times. The field of view of the entire area is preserved in the process. (D) FPM rendered images of CTCs trapped on filter. Between image d1 and d3, there is a height difference of 150 microns; FPM, unlike conventional microscopes, did not have to refocus during image acquisition. (E) More example images of CTCs trapped on the filter. The FPM approach provides a rich set of image data that we can use to render phase and bright-field images that can then be superimposed with fluorescence images taken with the same optical system.