# Chapter 2
# Affinity-Based Enrichment of Circulating Tumor Cells

**Zheng Ao, Richard J. Cote, and Ram H. Datar**

**Abstract** Study of CTC in cancer has always been hampered by its rare existence in blood. In this chapter, we discuss one of first principles employed to capture CTC from cancer patients' peripheral blood—the affinity-based enrichment of CTC. We briefly discuss the different technologies utilizing antibodies to capture CTC based on specific antigen expression. Then we address the downstream molecular and functional characterization of CTC by utilizing these technologies. We also discuss the limitations of affinity-based CTC enrichment.

**Keywords** Circulating tumor cells • Affinity-based cell capture • Molecular characterization of CTC • In vivo CTC culture

Z. Ao, Ph.D.
Sheila and David Fuente Graduate Program in Cancer Biology, University of Miami Miller School of Medicine, Miami, FL 33136, USA

R.J. Cote, M.D., F.R.C.Path., F.C.A.P.
Department of Pathology & Laboratory Medicine, University of Miami Miller School of Medicine, Miami, FL 33136, USA

Department of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, FL 33136, USA

Department of Pathology, Jackson Memorial Hospital, Miami, FL, USA

Dr. John T. Macdonald Foundation Biomedical Nanotechnology Institute, University of Miami Miller School of Medicine, Miami, FL 33136, USA

R.H. Datar, M.Phil., Ph.D. (✉)
Department of Pathology & Laboratory Medicine, University of Miami Miller School of Medicine, Miami, FL 33136, USA

Department of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, FL 33136, USA

Dr. John T. Macdonald Foundation Biomedical Nanotechnology Institute, University of Miami Miller School of Medicine, Miami, FL 33136, USA
e-mail: rdatar@med.miami.edu



R.J. Cote, R.H. Datar (eds.), *Circulating Tumor Cells*, Current Cancer Research,
DOI 10.1007/978-1-4939-3363-1_2

## 2.1 Background

Circulating Tumor Cells (CTCs) are tumor cells found in the peripheral blood of cancer patients, and the presence of these cells are considered the "real culprits" of metastatic disease that accounts for 90 % of all cancer-related deaths. Several studies were conducted in various types of cancers (breast, lung, colon, prostate, melanoma, etc.), presenting CTCs have proven prognosis value in these cancer types [1–3]. CTCs have also been shown to be valuable to predict drug-targeted therapy response in lung cancer patients [4]. As a result, there is increased interest in CTC research. In order to study CTCs these cells must be isolated and this can be challenging. In this chapter, we highlight one of the most widely used CTC-isolation techniques—affinity-based CTC capture. We discuss the principles behind the techniques, as well as explore emerging technologies employing these techniques. Lastly, we discuss the value and limitations of these various techniques.

## 2.2 Affinity-Based Enrichment of CTCs

As a rare population of cells in circulation, CTCs are difficult to study without the use of powerful technology that sets these cells apart from the surrounding and overwhelming population of leukocytes, red blood cells and other blood components. Among the various CTC enumeration technologies currently available, the use of the affinity-based isolation technique is the most widely adopted. These technologies utilize a unique antigen expression pattern on CTCs. The rationale behind the use of this type of CTC enumeration technology is based on unique antigen(s) expressed by CTC depending on their tissue of origin that are not shared with other normal components circulating in the blood. One of the most common examples among these is the use of the Epithelial Cell Adhesion Molecule (EpCAM) for CTC capture in epithelial cancer types. EpCAM is a transmembrane glycoprotein that is being expressed in cancers of epithelial origin, and not in normal components in circulation. Thus, antibodies against EpCAM can be used to selectively capture epithelial CTCs from peripheral blood. Here, we discuss several types of affinity-based technologies utilizing EpCAM or other tumor specific antigens in the capture of CTC cells (CellSearch and MagSweeper), as well as technologies that combine affinity-based capture with other physical parameters such as cell size (CTC-Chip and GEDI).

### *2.2.1 CellSearch*

To date, CellSearch is the gold standard in the CTC field. It is the first and still the only technology that is cleared by US Food and Drug Administration (FDA) for clinical CTC testing in metastatic prostate, breast, and colorectal cancers. Utilizing the

principle of magnetic field-based cell sorting, CellSearch captures CTCs by labeling them with ferrofluid nanoparticles functionalized with anti-EpCAM antibodies. These cells are then isolated via a magnetic field. Briefly, a blood sample is mixed with buffer and centrifuged, followed by aspiration of the plasma and buffer. Ferrofluid nanoparticles are then added to enrich CTCs with EpCAM expression. Post CTC enumeration, cells are labeled with Cytokeratin (Cytokeratin 8,18,19-phycoerythrin), which are epithelial markers to identify CTC; CD45 (CD45-allophycocyanin), which is a leukocyte marker for negative identification and DAPI, which labels nucleated cells. Results of harvested cells are then reviewed and CTCs are identified based on their staining profile (Cytokeratin positive, DAPI positive and CD45 negative) and morphology features [5].

The clinical utility of the CellSearch platform has been validated in various studies. The first milestone study in metastatic breast cancer patients indicates that, based on a cutoff of 5 CTCs detected by CellSearch system per 7.5 mL blood sample, metastatic breast cancer patients can be stratified to predict their disease outcome (Progression Survival and Overall Survival) [1]. This has also been validated in studies of other disease types such as prostate and colorectal cancer with a cutoff set at 5 and 3 CTCs respectively [2, 3].

### *2.2.2 MagSweeper*

MagSweeper is an automatic immunomagnetic separation technology for CTC enumeration. Blood samples are pre-diluted with a buffer and then incubated with magnetic-bead coated with anti-EpCAM antibodies to mark epithelial CTCs. Samples are then subjected to MagSweeper enumeration, during which magnetic rods covered with a plastic sheath are placed to sweep the sample well. After CTCs are captured on the rods, they are washed with a buffer and collected by external magnets that are located at the bottom of the well [6].

### *2.2.3 CTC-Chip*

CTC-Chip technology utilizes microposts functionalized with anti-EpCAM antibodies to capture CTCs. On the first generation of the CTC-Chip, 78,000 microposts were distributed on a 970-$mm^2$ surface and were functionalized with anti-EpCAM antibodies. Blood samples were processed through the CTC-Chip at a flow rate of 1–2 mL/h and captured CTCs were then identified via Cytokeratin positive, DAPI positive and CD45 negative staining. Although CTC-Chip technology is effective in capturing and analyzing CTCs, the volume that can be processed is limited to 2–3 mL [7]. This could be a major drawback if applied in clinical settings, as the standard blood sample collected is 7.5 mL. Reducing the sample volume could affect the sensitivity of the CTC capture. Thus, a second generation of the

CTC-Chip, HB-Chip, was designed with a Herringbone structure to disrupt the flow of blood and increase CTC collision with antibody-functionalized walls on the chip. This new design not only increases the volume of blood samples that can be processed using the chip, but also enables the capture of CTC clusters [8] that have shown to be clinically significant when analyzed by other CTC enrichment platforms [9]. The third generation of the CTC-Chip, CTC-iChip, utilizes a combination of hydrodynamic size-based cell sorting to deplete small cells in blood such as red blood cells and platelets. The inertial focusing microfluidics positions the cells into a single cell line and then either enriches the affinity-based labeled EpCAM positive CTCs or depletes the CD45 positive leukocytes with a magnetic field. This third generation of CTC-iChip not only allows for positive affinity-based enumeration of CTCs by magnetic activated cell sorting, but also allows for negative depletion of leucocytes for antigen agnostic CTC enumeration [10].

The clinical utility of the CTC-Chip still awaits large-scale clinical trial examination. A pilot study has indicated that by employing the CTC-Chip to analyze EGFR mutation in CTCs harvested from lung cancer patients, the responses for targeted therapies can be potentially predicted [4].

### *2.2.4 GEDI*

Geometrically enhanced differential immunocapture (GEDI) is a technology that captures prostate circulating tumor cells (PCTCs) based on their expression of prostate membrane-specific antigen (PSMA). The geometry of the device is designed to allow large prostate CTC's to collide onto obstacles, while smaller, non-target cells are displaced onto streamlines that do not collide with the microposts. This combination of affinity and size-based enrichment results in better capture efficiency and purity [11].

Worth noting, by switching the antibody used to functionalize the GEDI device, it is possible to achieve capture of CTCs from breast and gastric cancers using anti-HER2 antibody [12], pancreatic CTCs using anti-EpCAM and MUC1 antibodies [13], and mouse epidermal stem cells using anti-CD34 antibody [14].

## 2.3 Emerging Technologies for Affinity-Based Enrichment of CTCs

As a fast expanding community, CTC research has gained more and more attention in recent years. Thus, as previously described affinity-based CTC capture platforms are improving and a boom of fast-developing technologies are emerging. A few examples of these are discussed below:

### 2.3.1 NanoVelcro

NanoVelcro is a chip to capture CTCs based on their EpCAM expression. It is a device that is comprised of two parts; a patterned silicon nanowire substrate coated with anti-EpCAM antibodies and a polydimethylsiloxane (PDMS) chaotic mixer to generate flows that increase the collision between CTCs and capture substrates. Post CTC capture processes, CK/CD45/DAPI staining are executed to identify CTCs on the chip. A small, pilot cohort study of prostate cancer patients, utilizing the NanoVelcro chip, showed promising results for clinical applications [15]. Additionally, by grafting the NanoVelcro Chip with poly(*N*-isopropylacrylamide) (PIPAAm), a thermo- responsive polymer, captured tumor cells can be released via a temperature switch from 37 to 4 °C and cultured as demonstrated in model systems [16].

### 2.3.2 Graphene Oxide Nanosheets

This technology utilizes flower-shaped gold patterns to absorb graphene oxide (GO) and then functionalize the GO nanosheets with anti-EpCAM antibodies to capture CTCs in a PDMS chamber, and identify CTCs stained with CK/CD45/DAPI. The chamber-like platform also favors on-chip cell culture, tested with model systems where cultured tumor cells are spiked into blood, retrieved by GO nanosheets and cultured on chip [17].

### 2.3.3 VerIFAST

VerIFAST is a technology based on immiscible phase filtration (IPF) in which target cells are incubated with paramagnetic particles (PMPs), coated with EpCAM antibodies for capture and pulled with magnetic force into sequential chambers to achieve staining for EpCAM, Ki67, and Hoechst nuclear staining. The sieve chamber design in this technology can allow for an on-chip capture and staining workflow that minimizes substantial cell loss [18].

### 2.3.4 Immuno-microbubbles

This technology uses perfluorocarbon gas filled microbubbles conjugated with anti-EpCAM antibodies to capture CTCs. After CTCs are captured by the microbubbles, the layer of bubbles can be separated by centrifugation. The captured cell population is then subject to pan-CK/CD45/Hoechst staining for CTC identification [19].

### 2.3.5 GILUPI CellCollector

This emerging technology addresses the increasing need for in vivo CTC collection. This device collects CTCs by a functionalized and structured medical wire with anti-EpCAM antibodies that is inserted into the cubital vein of cancer patients for 30 min. This in vivo collection process, via this device, can analyze CTCs from 1.5 to 3 L of blood as compared with 7.5 mL blood analyzed by other ex vivo CTC assays [20].

## 2.4 Molecular and Functional Analysis of Circulating Tumor Cells Downstream of Affinity-Based CTC Enrichment

There has been a recent surge in the CTC capturing technology industry. Molecular and functional characterization of CTCs is becoming "hot-beds" for researchers seeking better utilization of CTCs in the clinical setting. Although the identification and quantification of CTCs alone can be effective in prediction of disease outcome [1–3], molecular and functional characterizations of CTCs are likely to increase the specificity of the CTC assay, leading to personalized targeted therapies.

Results from a recent clinical trial, SWOG 0500, indicates that chemotherapy treatment decisions based on elevated CTC numbers after a first cycle of chemotherapy does not benefit metastatic breast cancer patients [21]. Although the clinical utility of CTC numbers alone in determining treatment options needs further validation, these results exemplify the importance of, and demand for, CTC characterization. Here, we briefly discuss the characterization of CTCs enabled by the technologies we discussed above.

### 2.4.1 Gene Expression Analysis to Characterize CTC Post Affinity-Based Enrichment

Gene expression analysis is important in the prognosis and treatment of cancer as validated in primary tumor gene expression profiling experiments [22–24]. It is very probable that CTC gene expression analysis will more than likely provide critically necessary information for the management of diseases. It will also provide a critical research tool that will enhance the study of metastatic processes, tumor dormancy, tumor tropism, etc. Current attempts are trying to interrogate CTC gene expression profile at different levels.

Gene expression analysis at the mRNA level can be achieved on chip via RNA in situ hybridization (RNA ISH) as demonstrated by HB-CTC Chip to analyze human pancreatic CTC expression of *Wnt2* [25].

Off-chip mRNA analysis for products of tumor-specific genes can be analyzed by qRT-PCR or RNA-seq methods from pooled CTCs as demonstrated by data

obtained from CTC enrichment methods such as GEDI, GO nanosheets, and HB-CTC Chip [11, 17, 25]. However, due to contaminant leucocytes and other blood components captured nonspecifically by these affinity-based CTC enrichment methods, performing high-throughput mRNA analysis on both tumor and non-tumor-specific genes requires further isolation of pure population of CTCs in the post-capture process. This can be achieved by either the micropipetting of single cells (feasible by CTC-iChip technology) [10] or by microfluidics enabled by a single-cell analysis platform like Fluidigm (feasible by using MagSweeper technology) [26]. These single-cell analysis assays can be implemented to address issues raised regarding CTC heterogeneity that could be critical in the prognosis and treatment of cancer.

CTC gene expression profiles can also be interrogated at the protein level. This interrogation is already seen in most affinity-based CTC capture techniques. As an example, immunofluorescence (IF) staining is being employed to help distinguish CTCs from the background of leukocytes and other blood components. By adding an extra antibody or additional marker(s), it can be helpful in the qualification/quantification of protein marker expression levels when enabled by multi-spectrum fluorescence microscopy. CellSearch is a good example of technology that provides a user-defined channel tool that can be used in the characterization of CTCs [27]. There are several ongoing clinical trials are currently utilizing this feature to characterize CTCs for their Her2/Ki-67 expression and using the findings to determine the endocrine therapy used in metastatic breast cancer patients [28]. Multiplex immunofluorescence staining can also be used in the detection of fusion protein products such as TMPRSS2ERG fusion protein found in Castration-Resistant Prostate Cancer patients as demonstrated by the GEDI platform [11].

### *2.4.2 Genomic Analysis of CTC Post Affinity-Based Enrichment*

CTC genomics is another important aspect in the management of cancer. Analyzing mutations and chromosome rearrangements, as well as lineage tracing using Next Gen Sequencing (NGS) in CTCs, can provide valuable information in treatment selection and personalized therapy. It can also provide important biological-based information regarding the metastasis. DNA fluorescence in situ hybridization (FISH) is a common methodology utilized to analyze DNA point mutation and chromosomal rearrangement on chip. As demonstrated by HB-CTC Chip, FISH for Androgen Receptor (AR) gene locus can be performed on CTCs for copy number analysis [8]. Another efficient method for DNA mutation detection is PCR. This has been shown to be very successful in the detection of EGFR mutation from pooled CTCs isolated with CTC-Chip in lung cancer patients as described above [4]. DNA mutation detection by PCR has also proven to be an effective in the detection of KRAS and other gene mutations from CTC isolated by GILUPI CellCollector [20]. DNA mutation and chromosome translocations can also be detected on CTCs by RNA-ISH/IF techniques that can detect the mRNA or protein product of the mutation/fusion DNA [8, 11].

Next-Gen Sequencing is also being applied to analyze CTCs isolated by affinity-based methods. With a broader spectrum of CTC genomics, it can provide valuable information in treatment options, as well as insight in tumor evolution and the resistance development process. This is exemplified in a publication authored by Jens et al. addressing whole-exome sequencing of CTCs isolated from metastatic prostate cancer patients. Using a combination of automated cell picking with MagSweeper CTC isolation technology, they were able to achieve single cell whole-exome sequencing [29]. Xiaohui Ni et al. also achieved whole-exome sequencing using the CellSearch Platform to isolate CTCs from lung cancer adenoma patients. In this study, whole-exome sequencing was performed to analyze the copy number variation (CNV), and the patterns observed in the CTCs were found to be representative of metastatic tumors [30].

### *2.4.3 Functional Characterization of CTC Enabled by Affinity-Based Enrichment*

An emerging topic in CTC research is functional characterization of CTCs. This requires techniques that maintain cell viability in samples during the CTC capture process. To achieve optimal CTC viability, additional requirements for the handling, preserving, labeling, and shear pressure control should be implemented. It is worth noting that the CellSearch platform does not allow for viable CTC capture under harsh conditions. Some affinity-based platforms that allow for viable CTC capture are the CTC-iChip [10], GEDI [11], NanoVelcro chip [16], and Graphene Oxide nanosheets [17]. As a result, technologies that allow for viable CTC capture are driving CTC culture technology development and ex vivo drug treatment assays [11, 31] (Fig. 2.1).

| | Gene Expression Analysis | Genomic Analysis | Functional Characterization |
|---|---|---|---|
| CellSearch | Third marker immunofluorescence [28] | Copy Number Variation Analysis [31] | - |
| CTC-Chip | - | PCR [4] | - |
| HB-Chip | RNA ISH/ Pooled Cells RNA-Seq [25] | FISH/ Fusion transcript by RT-PCR [7] | - |
| CTC-iChip | Single Cell qRT-PCR [9] | - | CTC Culture/ Ex-vivo drug treatment [32] |
| MagSweeper | Single Cell qRT-PCR [27] | Whole Exome Sequencing [30] | - |
| GEDI | Pooled Cells RNA-seq [11] | Fusion protein by immunofluorescence [11] | Viable CTC Capture/ Ex-vivo drug treatment [11] |
| NanoVelcro | - | - | Viable CTC capture/ Culture in model system [16] |
| GO nanosheets | Pooled Cells qRT-PCR [17] | - | Viable CTC capture/ Culture in model system [17] |
| GILIPI CellCollector | - | PCR [20] | Viable CTC capture [20] |

**Fig. 2.1** Summary of molecular and functional analysis of circulating tumor cells downstream of affinity-based CTC enrichment

## 2.5 Limitations and Emerging Applications of Affinity-Based Enrichment of CTCs

### *2.5.1 Limitations*

Although affinity-based enrichment of CTCs has shown to be versatile and effective, the fact remains, that limitations related to this type of technology still exist. A major concern for affinity-based capture of CTCs is its utilization of antigen expression. Although EpCAM has shown to be an effective marker for CTC capture and is being used extensively in affinity-based CTC capture platforms, an increasing number of studies are also revealing its limitations. These limitations have been identified that CTCs with no or low EpCAM expression will be overlooked when EpCAM is used as the capture target. This was the case in a study featuring characterization of CTCs with potential for creating brain metastasis, this sub-group of CTCs were found to be EpCAM negative [32]. Another study conducted in metastatic breast cancer patients demonstrated how by combining of Cytokeratin and EpCAM expression for CTC detection, the sensitivity of CTC detection can be increased as compared with using solely EpCAM expression for CTC detection [33]. This could be due to the heterogeneity of CTC populations and the hypothesis that tumor cells undergo Epithelial–Mesenchymal Transition (EMT) and shed epithelial phenotypes such as EpCAM expression in order to increase invasion and metastatic capabilities [34]. It is important to note that the quality of the antibody and the position of the epitope that an antibody recognizes can also affect capture efficiency [35].

### *2.5.2 Emerging Applications for Affinity-Based Enrichment of CTCs*

Different strategies were attempted in order to overcome the potential bias introduced by EpCAM-based CTC capture. One strategy sought to increase the sensitivity of affinity-based CTC enrichment by using comprehensive panels to capture CTCs like using Cytokeratin in combination with EpCAM [33] or using a combination panel of EGFR, HPSE, ALDH1 [29]. Another strategy called for the negative depletion of non-tumor cells using an affinity-based isolation method instead of positively captures cells expressing certain markers. The $^{neg}$CTC-iChip is a notable example for this type of technology. By combining depletion of non-tumor cells using anti-CD45 and anti-CD15 antibodies with hydrodynamic cell-sorting based on cell size, the $^{neg}$CTC-iChip mode is able to isolate CTCs without biasing CTC capture with their antigen expression profiles.

## 2.6 Summary

Affinity-based enrichment serves as the first and most widely accepted principle employed in the isolation of CTCs. CellSearch technology, which utilizes this principle, is considered the gold standard in CTC research. Novel and evolving technologies seeking to increase capture efficiency are introducing state-of-the-art microfluidic designs. Numerous application potentials are being investigated to characterize CTCs captured with affinity-based principles at DNA, RNA and protein levels, as well as functional characterization of CTCs. Nevertheless, affinity-based CTC capture also faces some limitations with potential loss of sensitivity as a result of CTC heterogeneity, EMT process, and capture antibody efficiency. To overcome these limitations, technologies have been exploring various approaches to capture CTC that include the combination of panels of targets for CTC capture, combination of affinity-based capture with other principles such as size-based capture, and switching from positive capture to negative depletion of non-tumor cells in the sample.

## References

1. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Hayes DF (2004) Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 351(8):781–791
2. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Meropol NJ (2008) Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol 26(19):3213–3221
3. de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, Raghavan D (2008) Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res 14(19):6302–6309
4. Maheswaran S, Sequist LV, Nagrath S, Ulkus L, Brannigan B, Collura CV, Haber DA (2008) Detection of mutations in EGFR in circulating lung-cancer cells. N Engl J Med 359(4): 366–377
5. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, Terstappen LW (2004) Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res 10(20):6897–6904
6. Talasaz AH, Powell AA, Huber DE, Berbee JG, Roh KH, Yu W, Davis RW (2009) Isolating highly enriched populations of circulating epithelial cells and other rare cells from blood using a magnetic sweeper device. Proc Natl Acad Sci U S A 106(10):3970–3975
7. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, Toner M (2007) Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature 450(7173): 1235–1239
8. Stott SL, Hsu CH, Tsukrov DI, Yu M, Miyamoto DT, Waltman BA, Toner M (2010) Isolation of circulating tumor cells using a microvortex-generating herringbone-chip. Proc Natl Acad Sci U S A 107(43):18392–18397
9. Hou JM, Krebs MG, Lancashire L, Sloane R, Backen A, Swain RK, Dive C (2012) Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer. J Clin Oncol 30(5):525–532
10. Ozkumur E, Shah AM, Ciciliano JC, Emmink BL, Miyamoto DT, Brachtel E, Toner M (2013) Inertial focusing for tumor antigen-dependent and -independent sorting of rare circulating tumor cells. Sci Transl Med 5(179):179ra47

11. Kirby BJ, Jodari M, Loftus MS, Gakhar G, Pratt ED, Chanel-Vos C, Giannakakou P (2012) Functional characterization of circulating tumor cells with a prostate-cancer-specific microfluidic device. PLoS One 7(4):e35976
12. Galletti G, Sung MS, Vahdat LT, Shah MA, Santana SM, Altavilla G, Kirby BJ, Giannakakou P (2014) Isolation of breast cancer and gastric cancer circulating tumor cells by use of an anti HER2-based microfluidic device. Lab Chip 14(1):147–156
13. Thege FI, Lannin TB, Saha TN, Tsai S, Kochman ML, Hollingsworth MA, Kirby BJ (2014) Microfluidic immunocapture of circulating pancreatic cells using parallel EpCAM and MUC1 capture characterization, optimization and downstream analysis. Lab Chip 14(10):1775–1784
14. Zhu B, Smith J, Yarmush ML, Nahmias Y, Kirby BJ, Murthy SK (2013) Microfluidic enrichment of mouse epidermal stem cells and validation of stem cell proliferation in vitro. Tissue Eng Part C Methods 19(10):765–773
15. Lu YT, Zhao L, Shen Q, Garcia MA, Wu D, Hou S et al (2013) NanoVelcro Chip for CTC enumeration in prostate cancer patients. Methods 64(2):144–152
16. Hou S, Zhao H, Zhao L, Shen Q, Wei KS, Suh DY, Yu HH (2013) Capture and stimulated release of circulating tumor cells on polymer-grafted silicon nanostructures. Adv Mater 25(11):1547–1551
17. Yoon HJ, Kim TH, Zhang Z, Azizi E, Pham TM, Paoletti C, Nagrath S (2013) Sensitive capture of circulating tumour cells by functionalized graphene oxide nanosheets. Nat Nanotechnol 8(10):735–741
18. Casavant BP, Guckenberger DJ, Berry SM, Tokar JT, Lang JM, Beebe DJ (2013) The VerIFAST an integrated method for cell isolation and extracellular/intracellular staining. Lab Chip 13(3):391–396
19. Shi G, Cui W, Benchimol M, Liu YT, Mattrey RF, Mukthavaram R, Simberg D (2013) Isolation of rare tumor cells from blood cells with buoyant immuno-microbubbles. PLoS One 8(3):e58017
20. Saucedo-Zeni N, Mewes S, Niestroj R, Gasiorowski L, Murawa D, Nowaczyk P, Lücke K (2012) A novel method for the in vivo isolation of circulating tumor cells from peripheral blood of cancer patients using a functionalized and structured medical wire. Int J Oncol 41(4):1241–1250
21. Smerage JB, Barlow WE, Hayes DF, Winer EP, Leyland-Jones B, Srkalovic G, Hortobagyi GN (2013) Abstract S5-07 SWOG S0500—a randomized phase III trial to test the strategy of changing therapy versus maintaining therapy for metastatic breast cancer patients who have elevated circulating tumor cell (CTC) levels at first follow-up assessment. Cancer Res 73(24 Suppl):S05–S07
22. van't Veer LJ, Dai H, Van De Vijver MJ, He YD, Hart AA, Mao M et al (2002) Gene expression profiling predicts clinical outcome of breast cancer. Nature 415(6871):530–536
23. Lapointe J, Li C, Higgins JP, van de Rijn M, Bair E, Montgomery K et al (2004) Gene expression profiling identifies clinically relevant subtypes of prostate cancer. Proc Natl Acad Sci U S A 101(3):811–816
24. Wang Y, Jatkoe T, Zhang Y, Mutch MG, Talantov D, Jiang J et al (2004) Gene expression profiles and molecular markers to predict recurrence of Dukes' B colon cancer. J Clin Oncol 22(9):1564–1571
25. Yu M, Ting DT, Stott SL, Wittner BS, Ozsolak F, Paul S et al (2012) RNA sequencing of pancreatic circulating tumour cells implicates WNT signalling in metastasis. Nature 487(7408): 510–513
26. Powell AA, Talasaz AH, Zhang H, Coram MA, Reddy A, Deng G et al (2012) Single cell profiling of circulating tumor cells transcriptional heterogeneity and diversity from breast cancer cell lines. PLoS One 7(5):e33788
27. Lowes LE, Hedley BD, Keeney M, Allan AL (2012) User-defined protein marker assay development for characterization of circulating tumor cells using the Cell Search® system. Cytometry A 81(11):983–995
28. Janssen Diagnostics, LLC; Janssen Diagnostics, LLC. Characterization of circulating tumor cells (CTC) from patients with metastatic breast cancer using the CTC-Endocrine Therapy Index (COMETI P2). In ClinicalTrials.gov [Internet]. Bethesda (MD) National Library of

Medicine (US). 2012- [cited 2014 June 18]. Available from http//www.clinicaltrials.gov/ct2/show/NCT01701050 NLM Identifier NCT01701050
29. Lohr JG, Adalsteinsson VA, Cibulskis K, Choudhury AD, Rosenberg M, Cruz-Gordillo P et al (2014) Whole-exome sequencing of circulating tumor cells provides a window into metastatic prostate cancer. Nat Biotechnol 32(5):479–484
30. Ni X, Zhuo M, Su Z, Duan J, Gao Y, Wang Z et al (2013) Reproducible copy number variation patterns among single circulating tumor cells of lung cancer patients. Proc Natl Acad Sci U S A 110(52):21083–21088
31. Yu M, Bardia A, Aceto N, Bersani F, Madden MW, Donaldson MC et al (2014) Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. Science 345(6193):216–220
32. Zhang L, Ridgway LD, Wetzel MD, Ngo J, Yin W, Kumar D et al (2013) The identification and characterization of breast cancer CTCs competent for brain metastasis. Sci Transl Med 5(180):180ra48
33. Deng G, Herrler M, Burgess D, Manna E, Krag D, Burke JF (2008) Enrichment with anti-cytokeratin alone or combined with anti-EpCAM antibodies significantly increases the sensitivity for circulating tumor cell detection in metastatic breast cancer patients. Breast Cancer Res 10(4):R69
34. Gorges TM, Tinhofer I, Drosch M, Röse L, Zollner TM, Krahn T, von Ahsen O (2012) Circulating tumour cells escape from EpCAM-based detection due to epithelial-to-mesenchymal transition. BMC Cancer 12(1):178
35. Antolovic D, Galindo L, Carstens A, Rahbari N, Büchler MW, Weitz J, Koch M (2010) Heterogeneous detection of circulating tumor cells in patients with colorectal cancer by immunomagnetic enrichment using different EpCAM-specific antibodies. BMC Biotechnol 10(1):35

http://www.springer.com/978-1-4939-3361-7

Circulating Tumor Cells
Cote, R.J.; Datar, R.H. (Eds.)
2016, XXIV, 333 p. 31 illus., 29 illus. in color., Hardcover
ISBN: 978-1-4939-3361-7