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REVIEW ARTICLE

Micro- and nanotechnology approaches for capturing circulating tumor cells

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Abstract Circulating tumor cells (CTC) are cells that have detached from primary tumors and circulate in the bloodstream where they are carried to other organs, leading to seeding of new tumors and metastases. CTC have been known to exist in the bloodstream for more than a century. With recent progress in the area of micro- and nanotechnology, it has been possible to adopt new approaches in CTC research. Microscale and nanoscale studies can throw some light on the time course of CTC appearance in blood and CTC over-expression profiles for cancer-related markers and galvanize the development of drugs to block metastases. CTC counts could serve as endpoint biomarkers and as prognostic markers for patients with a metastatic disease. This paper reviews some of the recent researches on using micro- and nanotechnology to capture and profile CTC.

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1 Circulating tumor cells

It has been known for a long time that cells detach from primary tumors and reach different organs through the body's circulating system, therefore giving them the name "circulating tumor cells" (CTC). It was in 1869 that Thomas Ashworth first observed circulating tumor cells in the blood of a man with metastatic cancer using a microscope (Ashworth 1869). He postulated that "cells identical with those of the cancer itself being seen in the blood may tend to throw some light upon the mode of origin of multiple tumors existing in the same person". A thorough comparison of the morphology of the circulating cells to tumor cells from different lesions led Ashworth to conclude that "One thing is certain, that if they (CTC) came from an existing cancer structure, they must have passed through the greater part of the circulatory system to have arrived at the internal saphena vein of the sound leg". Since the 1950s, many studies have demonstrated the presence of CTC in blood and some of them even shed light that there may be prognostic value on the circulating tumor cells in patients with breast cancer (Colombo et al. 1959; Wilson 1959; Rohmsdahl et al. 1960; Soost 1960; Graeber 1961; Rohmsdahl et al. 1961; Saito 1961; Wuest and Birk 1962; Candar et al. 1962). Only in the 1990s did clinicians understand the utility of CTC for diagnosis due to a study that showed the presence of primary tumor cells lodged in

the bone marrow before metastases become evident (Shpall et al. 1993; Weiss and Geduldig 1991; Brugger et al. 1994; Spall and Jones 1994). However, the capture and quantification of such CTC in blood has faced difficult technological hurdles because these cells are rare. CTC are no bigger than leukocytes; their low number, typically one to ten per milliliter makes their detection very difficult using traditional techniques such as microscopy. The shedding of CTC into blood is a discontinuous process and the detected CTC are often heterogenous (Paterlini-Brechot and Benali 2007; Mocellin et al. 2006; Jacob et al. 2007). Most of the CTC that are shed into the blood will never colonize any organs because the vast majority of the CTC will be eliminated from the blood circulation (Gerges et al. 2010). However, once even a few cells manage to establish conditions for growth at distant sites, development of tumors at those sites seeded by the initial cells may become very efficient, resulting in poor prognosis for the patients (Gerges et al. 2010). In carcinomas, which are solid tumors derived from epithelial tissues, representing 80% of all diagnosed cancer cases, CTC can often be distinguished by epithelial lineage markers, which serve to identify CTC and 'occult metastasis' even at the single-cell level, especially in the blood and bone marrow of cancer patients. CTC may affect cancer prognosis years before the onset of overt metastasis, improve the risk assessment, and help identify patients in need of treatment (Zieglschmid et al. 2005; Braun et al. 2005; Braun and Naume 2005). These cells themselves may potentially provide novel therapeutic targets. To reflect these developments, CTC detection has been introduced into the international tumor staging systems, and their use as tumor markers in breast cancer has been recommended by the American Society of Clinical Oncology in 2007 (Singletary et al. 2003; Singletary and Greene 2003; Hermanek et al. 1999).

Understanding CTC may be the first step to block metastases and therefore could have a positive impact on patient survival and management in the clinic. Currently, the best data supporting the use of CTC in cancer patient management exist for patients with primary breast cancer, whereas other disease sites are being studied using novel technologies. Several large studies have shown that lodging of tumor cells in the bone marrow of patients whose cancers tend to spread to the bone (breast, prostate) is associated with poor prognosis (Slade and Coombes 2007). In some cases, the detection of tumor cells in the bone marrow had a superior prognostic value or clinical significance compared to detection of CTC in blood (Pierga et al. 2004). However, bone marrow biopsy is invasive and is not suitable for repeated or routine implementation in the clinic. CTC counts could be used to serve as prognostic endpoint biomarkers that can be done in a minimally invasive way by repeated blood draws. With the advent of micro- and nanotechnology, it has become possible to create materials, devices, and systems at the level of atoms, molecules, and supramolecular structures. Micro- and nanotechnology approaches are gaining momentum in being able to capture CTC with high efficiency in blood.

2 Cancer nanotechnology and circulating tumor cells

Nanotechnology encompasses the creation, manipulation, and utilization of materials, devices, and systems at the level of atoms, molecules, and supramolecular structures. Formal definitions of nanotechnology include anywhere between 1 and 1,000 nm in size. With smaller size, new physical properties emerge, and therefore new techniques are required to make nanomaterials and characterize them. Cancer-related examples of nanotechnologies include injectable drug delivery nanovectors such as liposomes for the therapy of breast cancer (Ferrari 2005 Mar; Park 2002); biologically targeted, nanosized magnetic resonance imaging (MRI) contrast agents for intraoperative imaging in the context of neuro-oncological interventions (Kircher et al. 2003; Neuwalt et al. 2004); and novel, nanoparticle-based methods for high-specificity detection of DNA and protein (Nam and Mirkin 2004). The use of micro- and nanotechnology to detect and capture CTC in blood is a relatively new area of research. CTC are no larger than leukocytes and isolation of CTC from blood components is challenging owing to their low concentration. Most of the CTC detection systems available today use some kind of an enrichment technique to improve detection sensitivity. However, they also lose CTC in the process of enrichment due to multiple batch purification steps.

The ideal detection system should be sensitive, should have high specificity, and should be small enough to be portable in the clinical setting. Further, the ideal CTC detector should be able to count the number of CTC at a rapid pace (<1 h) and should have minimum sample preparation or purification steps. Enrichment steps should also be avoided to minimize the loss of CTC. Finally, the ideal CTC system should also be able to decipher biological information such as different types of biomarkers, genetic mutations in tumor DNA, and any other information that reflects the biological processes occurring during metastasis. Therefore, the development of an ideal CTC detector is still far from reality. The small size of nanoparticles, ability to create nanoparticle contrast agents, and with a suitable detection methodology, nanotechnology can pave the way for CTC detection and monitoring in vivo.

CTC detection methods can be classified into two types. These are (1) immunological assays that use monoclonal antibodies against cell surface antigens and (2) PCR-based methods that detect tumor-specific DNA or RNA. Immu-

nological methods have been widely used for CTC detection. The choice of appropriate markers is a challenge as antigens exclusively expressed by CTC and not shared by other circulating non-tumor or blood cells are scarce. Antibodies specific to epithelial antigens such as cytokeratin and EpCAM are the most widely used markers for epithelial tumor cell detection (Mostert et al. 2009). Indeed while CK19 presence in the blood has been correlated with higher levels of metastasis and worse prognosis (Kircher et al. 2003), loss of CK19 has also been documented in cancer cells and thus may generate false negative results (Jacob et al. 2007). In addition, the percentage of CK-positive cells in normal controls ranges from 0% to 20% in instances of non-specific binding of non-tumoral cells or in instances of specific binding to circulating epithelial cells, which are present due to trauma or inflammation within the body. Organ-specific markers, including prostate-specific antigen, carcinoembryogenic antigen (CEA), or Her-2 have also been used. However, false negative/positive results are also possible as these markers are not present in all tumor cells (only up to 30% of cancer cells carry Her-2 in Her-2-positive breast cancer) or are not entirely organ specific. More recently, CTC detectors specific for prostate-specific membrane antigen (PSMA) have been created to detect prostate cancer cells with high efficiency. Several immunofluorescence-based technologies are being used to improve the threshold of detection (Krivacic et al. 2004; He et al. 2007). Enrichment methods with anti-cytokeratin or combinations of anti-cytokeratin and anti-EpCAM antibodies have been shown to improve the enrichment process for CTC that have low EpCAM expression (Deng et al. 2008).

3 CTC detectors

3.1 Conventional techniques

Several technologies are available for the detection of CTC in human blood as shown in Fig. 1. Traditionally, density gradient centrifugation is the method that has been used for removing CTC for microscopy (Baker et al. 2003; Lara et al. 2004; Pantel and Brakenhoff 2004). Heavier components in the blood sink to the bottom while the lighter mononuclear components including tumor cells float to the top. These are then transferred to a slide and stained for epithelial markers such as EpCAM to detect CTC. A trained pathologist must examine the slides for CTC. This process is time-consuming, can take days to obtain a report for one sample, and is subject to false positives and negatives depending on the skill of the analyst. Moreover, density gradient centrifugation has a recovery rate no better than 70%. The downfall of using many of these gradient liquids is that whole blood tends to mix with the gradient if not centrifuged immediately; therefore, total separation is easily interrupted.

Isolation of CTC using polycarbonate filters have been demonstrated (Pinzani et al. 2006; Vona et al. 2000, 2002, 2004; Kahn et al. 2004). It is an inexpensive and simpler form of enrichment and capture of CTC. It exploits the fact that CTC are significantly larger than surrounding blood cells. The polycarbonate filters have track etching that results in the random placement of pores. This results in low density and often results in the fusion of two or more pores together. Although the claimed efficiency of capture is 50–60%, the methods are nevertheless questionable as it is quite possible to mix CTC with leukocytes as they have similar sizes. CTC are also not always greater than 8–10 μm, and one can therefore produce false positive and false negative results using this approach. One of the new devices that is a modified form of polycarbonate filters is the paralyne C microfilter assembly for the capture of CTC (Zheng et al. 2007). Parylene C offers several distinctive properties for CTC capture. It is the highest USP class IV biocompatible polymer for implementation, and thus biofouling is expected to be minimal for parylene C. It has excellent mechanical properties that allow it to be stretched up to 200% (Zheng et al. 2007). It has a high degree of transparency in the UV and visible range, making it possible to observe and stain CTC directly on the filter without having to transfer cells to a glass slide. This translates to minimal cell loss. While the use of parylene C for CTC has shown recovery of 90%, it makes the same assumption as the polycarbonate filter-based devices that CTC are significantly larger than red blood cells, which is still questionable. So, this technique suffers from the same drawbacks as the polycarbonate filter approach, making it prone to produce false positive or negative results.

3.2 Micrometer-scale technologies for capturing CTC

CellSearch (Veridex) is the first widespread CTC detector that has been approved by the Food and Drug Administration (Cristofanilli et al. 2004). It works for epithelial cancers, namely, breast, colon, and prostate. The system is based on the enumeration of epithelial cells, which are separated from the blood by antibody-coated magnetic beads and identified using fluorescently labeled antibodies against cytokeratin, and a fluorescent nuclear stain. A total of 177 breast cancer patients were enrolled and tested for their CTC counts over a period of 2 years. Outcomes were assessed according to the levels of CTC at baseline, before the patients started a new treatment for metastatic disease. It was found that patients in a training set with levels of CTC equal to or higher than five CTC per 7.5 ml of whole blood, as compared to those with fewer than five CTC per 7.5 ml, had a shorter median progression-free survival (2.7 months

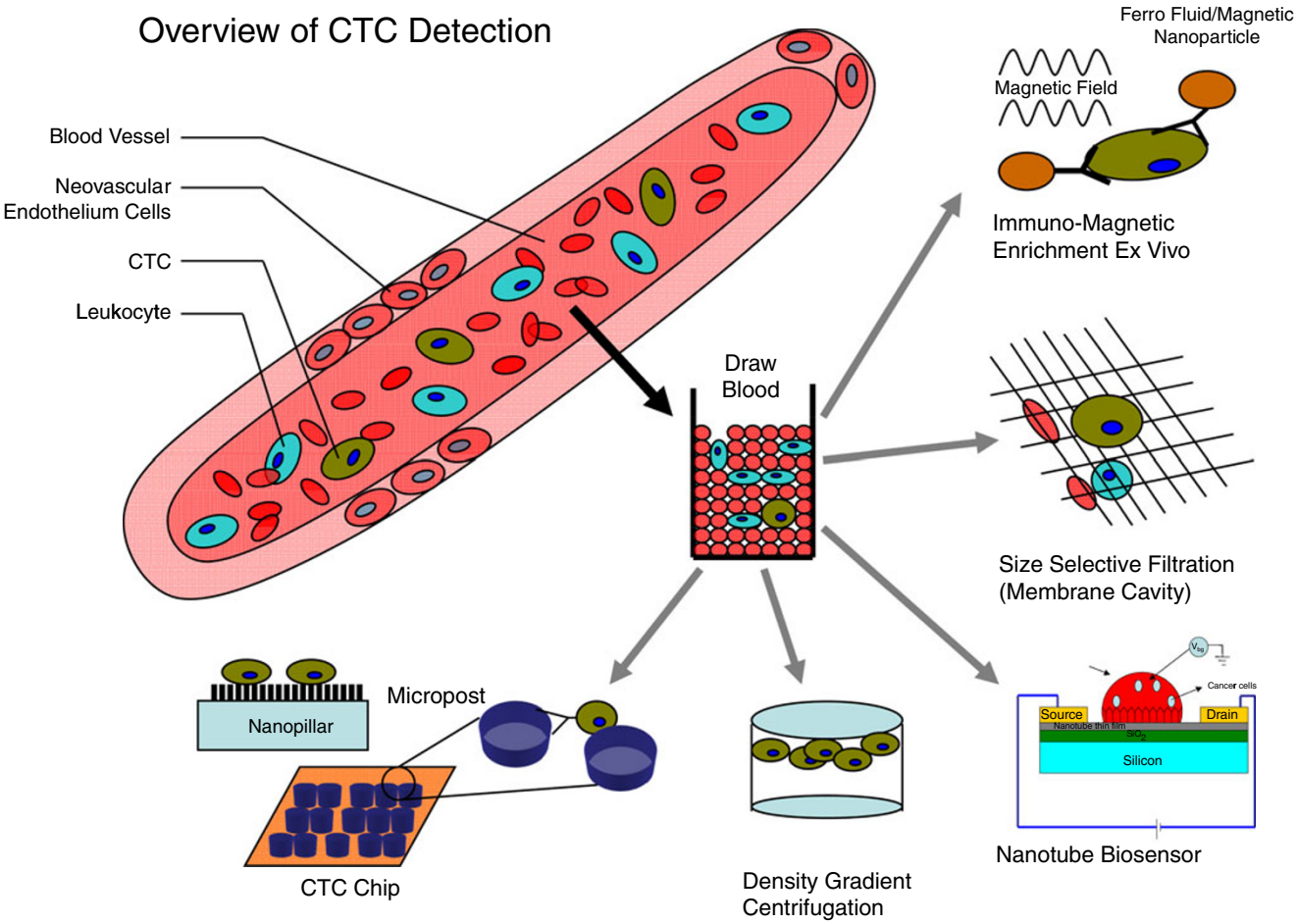

Fig. 1 Schematic of current approaches for the detection of circulating cancer cells

vs 7.0 months P<0.001) and shorter overall survival (10.1 months vs 18 months P<0.001). A total of 68% of the tumors were positive for estrogen receptor or progesterone receptor or both, 30% were negative for both, and 26% were positive for Her2-neu. It was found using the multivariate Cox proportional hazards regression model that levels of CTC at baseline and at the first follow-up visit were the most significant predictors of progression-free and overall survival. The study showed that, among the various groups of patients, the level of CTC was significantly different only in those patients who received hormone therapy or immunotherapy or both as compared to patients starting chemotherapy. The prognostic implications of this study are that elevated levels of CTC in patients present opportunities to stratify patients for investigational studies. Systems such as CellSearch suffer from several drawbacks. Firstly, multiple steps of batch purification and enrichment result in CTC loss. The actual number of CTC might be much higher to start with in each patient group. Secondly, it might be difficult to capture cells that do not express EpCAM, possibly because the cells have undergone epithelial mesenchymal transformation (EMT), which makes the cells less susceptible to stick to the antibodies as they break free into the blood circulation. EpCAM methods are also not useful for non-epithelial cancers such as sarcomas. Nevertheless, this is the only FDA-approved CTC detector currently on the market.

The CTC chip is an exciting technology that uses passive microfluidic sorting of blood cells (Nagrath et al. 2007). The CTC chip has 78,000 micro-posts that are etched in silicon. Antibodies such as anti-EpCAM are functionalized on the surfaces of the micro-posts. Anti-EpCAM provides the specificity for CTC capture from unfractionated blood as it is overexpressed in epithelial cells and is absent in heamatologic cells. The CTC chip measured the number of CTC in peripheral blood from patients with metastatic lung, prostate, pancreatic, breast, and colon cancer in 115 of 116 samples with a range of five to 1,281 CTC per milliliter and approximately 50% purity. The CTC chip efficiency depends on the flow velocity of the blood because it influences the duration of the cell–micropost contact and the shear force, which must be sufficiently low to ensure maximum cell–micropost attachment. The flow rates are extremely low, of the order of 1.0 ml/h. With such a small flow rate, the CTC

chip takes 6–8 h of sorting time for one sample of 10 ml of patient blood, followed by confocal microscopy. The yield of the CTC capture goes to less than 20% at 3.0 ml/h (Nagrath et al. 2007). Another drawback of the CTC chip is that it targets only EpCAM and therefore cannot capture cells that have undergone EMT. Finally, the CTC chip is less useful for sarcomas which cannot be captured using EpCAM methods.

While most of the devices for CTC capture have focused on breast cancer, highly efficient capture and enumeration of low-abundance prostate cancer cells using prostate-specific membrane antigen aptamers immobilized to a polymeric microfluidic device was demonstrated recently (Dharmasiri et al. 2009). The device consisted of 51 ultra-high aspect ratio curvilinear channels with width similar to prostate cell dimensions. By functionalizing the surface of the channels with a high density of PSMA-specific aptamers and using a flow velocity of 2.5 mm/s, the recovery of LnCAP cells in blood spiked with 20 cells per milliliter was found to be 90%. The total time of capture for 1 ml of sample was reported to be 29 min.

Size-selective filtration of blood to capture CTC was described above in conventional methods of CTC capture. A new device that was reported this year used size-selective micro-cavity array for rapid and efficient detection of CTC (Hasokawa et al. 2010). The size-selective microcavity array was made of nickel electroforming. The micro-cavities were fabricated with diameters of 8, 9, 10, and 11 μm at the top surface. The distance between each cavity was 60 μm and a total of 10,000 cavities were arranged in 100×100-μm arrays. A negative pressure was introduced into the chamber through a peristaltic pump to enable cell entrapment into the cavities. There were ten to 100 NCI-H358 lung cancer cells spiked into 1 ml of blood. It was reported that the device successfully captured 97% of the lung carcinoma cells. Further, the device was able to detect EpCAM-negative tumor cells with an efficiency of 80%. It was found that the maximum recovery of the cells were at 9-μm cavity size. The technique is similar to the parylene-C-coated device except that the pores are more optimized and the conical shape of the pores makes it more effective to avoid clogging of blood cells. While this is definitely an efficient device for trapping cells, the mechanism of cell capture relying purely on size effects is still questionable. It is possible that cancer cells can also deform and get out of the cavities similar to red blood cells or leukocytes. However, this method is definitely cost-effective compared to devices that use antigen–antibody interactions which tend to be more expensive.

3.3 Nanotechnology methods for CTC detection

Nanoscale materials can improve the scale of CTC detection in many ways. First, nanoscale materials alter the surface interaction of cells to the substrate. Surface topography of nanoscale materials can lead to the adherence of cells or even cellular differentiation. For example, it has been shown that neuronal cells grown on silicon substrates with average surface roughness between 20 and 100 nm promoted longevity as well as better cell adhesion to the substrates that could be used conveniently for recording neuronal activity in cultures (Fan et al. 2002 Oct 15; Khan et al. 2005 Jun; Kriparamanan et al. 2006 Jul). Nanomaterials such as carbon nanotubes promote cellular differentiation. In a recent study, 2D thin film scaffolds composed of biocompatible polymer-grafted carbon nanotubes (CNTs) were shown to selectively differentiate human embryonic stem cells into neuronal cells while maintaining excellent cell viability (Chao et al. 2009). Fluorescence imaging showed that the neuron differentiation efficiency of poly(acrylic acid)-grafted CNT thin films was significantly greater than that on poly(acrylic acid) thin films. When compared with the conventional poly-L-ornithine surfaces, a standard substratum commonly used for neuron culture, this new-type thin film scaffold shows enhanced neuron differentiation. No noticeable cytotoxic effect difference was detected between these two surfaces. The surface analysis and cell adhesion study have suggested that CNT-based surfaces can enhance protein adsorption and cell attachment (Chao et al. 2009). Three-dimensional nano-structured substrates could alter the surface adhesion of the cells and aid in the capture of CTC in blood. A recent report showed the use of nano-pillars fabricated using top-down manufacturing techniques that are functionalized with EpCAM antibodies to capture CTC (Wang et al. 2009). The three-dimensional substrates of nano-pillars are made out of silicon. First, densely packed nanopillars of about 100–200 nm in size were fabricated on silicon substrates using wet chemical etching. The length of these chemically etched nanopillars can be controlled by applying different etching times. After the preparation of silicon nano-pillars, *N*-hydroxysuccinimide functionalization chemistry is used to introduce streptavidin onto the surfaces of the nano-pillars substrates. Biotinylated anti-EpCAM antibodies were introduced onto the streptavidin-bonded substrates for conjugation of antibodies prior to the cell capture experiments. To test the efficiency of cell capture of the silicon nano-pillars, a cell suspension of an EpCAM-positive cell line, MCF7 cells, was introduced into the silicon nanopillars and incubated for an hour. As a control, flat silicon surfaces were used to compare with the nano-pillar surfaces. It was found that the flat silicon substrates only captured between 4% and 14% of the cells vs. nano-pillars which captured 45–65% of the cells. This suggests that 3D nano-pillars are responsible for enhanced cell capture. It was also found that the captured cells in the silicon nano-pillars exhibited morphological differences with nanoscale cellular protrusions and were more elongated than cells captured on the flat silicon surfaces. A series of artificial CTC blood samples were prepared by spiking MCF7 cells

into rabbit blood followed by fluorescence microscopy. It was found that the nano-pillars were able to capture cells at efficiencies of >40% compared to commercially available technologies (Wang et al. 2009). While the nano-pillar substrates offer an efficient form of cell capture, the clinical relevance of such devices has yet to be established. More research on why cancer cells alone attach to the nano-pillar and elongate as opposed to leukocytes and red blood cells has yet to be determined.

One of the most interesting nanoscale materials that are actively researched today is carbon nanotubes for electronics, sensing, and actuating. Most of the atoms in single-wall carbon nanotubes are surface atoms and any change therefore in the surface can affect their electron transport properties. The capture of CTC using nanotubes is highly novel as it uses the biological interactions on the surface of the nanotubes to alter the electron transport properties of the carbon nanotube (Shao et al. 2008). The reported device uses carbon nanotubes as nanoscale transducing elements to capture CTC in blood. Small bundles of carbon nanotubes were patterned between pairs of gold electrodes. Initially, two columns composing a ten-element array were created for looking at the interactions of cells on nanotube surfaces that are coated with IGF1R and Her2 antibodies. A non-specific IgG was used as an antibody control, and MCF10A non-tumorigenic breast cells were used as cellular controls. BT474 breast cancer cells that overexpress Her2 and MCF7 breast cancer cells that overexpress IGF1R were spiked into blood at the level of 10,000 cells in 1 μl of human donor blood. The electrical signals were recorded as a function of time. It was found that unique signatures were obtained for specific and non-specific interactions. Optical microscopy of the devices showed that, while there may be 10,000 cells spiked in blood, only a single cell attaches to a single device junction to create the change in electrical signal. This was attributed to the change in current between MCF7 cells, BT474 cells, and MCF10 A cells. These results were reproducible and showed that the electronic effects of semiconducting nanowire or nanotube could in principle be used to capture CTC. Further, the device did not foul in blood; continuous recording of electrical signals could be obtained with ease. While the devices are highly sensitive for capturing specific events, the clinical utility of such devices has yet to be established for effective capture of CTC in blood. An integrated system consisting of arrays of sensors inside a microfluidic trap would be useful for the capture of single CTC in blood. Further, this device could also be useful for the profiling of receptor overexpression in fine needle aspirates for rapid liquid biopsy.

3.4 Nanotechnology-based in vivo detection

The size, chemical and biological compatibility, and unique physical properties of nanoscale materials can be exploited for in vivo detection of CTC. This would definitely be a quantum leap in technological development for monitoring CTC, the time of release of CTC in blood, and monitoring therapeutic efficacies. A recent report has shown the efficacy of magnetic enrichment and photoacoustic detection of CTC in vivo in a mice model (Galanzha et al. 2009). It takes the approach that ex vivo methods are not sensitive enough owing to the limited amount of blood sample volume to monitor the onset of disease progression. Therefore, continuous monitoring of blood in patients would be highly useful to detect CTC in peripheral blood and also to monitor the time of CTC release in blood from a primary tumor. First, antibodies specific to a receptor in cancer cells were conjugated to magnetic nanoparticles. These conjugated magnetic nanoparticles captured CTC in blood, thereby enabling invivo magnetic enrichment. A magnet was attached to the skin of the mouse to enable enrichment of the magnetic nanoparticles that are bound to the CTC. This improved the photoacoustic detection from infrequent flashes to continuous signal. To improve sensitivity, gold-plated carbon nanotubes conjugated with folic acid were used as a second contrast agent for photoacoustic imaging. To detect CTC originating from the primary tumor, 5×10^6 MDA-MB-231 cells were inoculated subcutaneously in mice. At 2, 3, and 4 weeks of tumor development, a cocktail of the conjugated nanoparticles was injected intravenously into the circulation. Two-color photoacoustic detection of CTC at 20 min after injection showed that the ratio of the number of CTC in mouse ear to those in abdominal vessel increased from 0.9±0.3/6±2.1 at 2 weeks to 7.2±0.3/26±2.1 at 3 weeks and to 15.1±2.7/47±6.4 at 4 weeks. It was found that the number of CTC that appeared roughly correlated with the stage of primary tumor progression. This technique of using duplex molecular targeting of CTC with iron nanoparticles followed by their capture using dual magnetic–photoacoustic flow cytometry will be highly useful clinically, provided they are found to be tolerated in humans. In the clinic, patients may be made to wear a magnetic jacket for acquiring continuous photoacoustic signals as compared to discontinuous ones. If proved successful, this in vivo technology can be a powerful monitor as to the onset of disease progression and therapeutic monitoring in vivo. Further, photothermal killing of cancer cells using laser would prove useful to eradicate cells associated with primary tumor and block metastases.

4 Clinical relevance of CTC detectors

CTC counts could have high clinical relevance in a number of areas in the clinical management of cancer. First, CTC can survive chemotherapy and therefore can be a predictive marker for the effectiveness of treatment. The Veridex study

on 177 breast cancer patients showed that the very short median progression-free survival in patients with elevated levels of CTC at the first follow-up visit suggests that these patients were receiving ineffective therapy (Vona et al. 2004). Nanotechnology methods such as nanopatterned surfaces could potentially capture a single CTC in 10 ml of blood, thereby enabling detection of CTC upon their release into the blood stream. Secondly, CTC detection at low numbers could be a prognostic marker for disease progression. Surgery or suitable therapy can be administered to block metastases. Here again, surgery based on nanotechnology methods could be minimally invasive compared to chemo- or radiotherapy. Past techniques using nano-shells and carbon nanotubes have shown selective thermal destruction of cancer cells using benign NIR light (Hirsch et al. 2006 Jan; Kam et al. 2005; Panchapakesan et al. 2005; Shao et al. 2007). It is becoming more recognized that CTC counts could indicate early versus late stage of the disease and correlate with survival. Thirdly, the biological aspects of CTC have still not been thoroughly delineated. Profiling of CTC for various surface markers and quantifying their degree of overexpression can provide guidance for effective therapy. Again, nanotechnology-based electronic, optical, and magnetic detectors could be used to develop handheld molecular profilers that could stratify patients in minutes based on their immunohistological signatures. Nanotechnology-based methods can also galvanize customized drug cocktails to block metastases depending on the patients' cellular profiles. Finally, the genetic make-up of CTC is still a not well-understood phenomenon and could throw some light into the EMT process. CTC have recently shown to exhibit stem cell-like properties (Marx 2007). Tumors grown using CTC in a mice model grew twice as large in the same 3-week time period compared to normal tumor growth from the original cell line (Kaiser 2010). Further, CTC have also been known to colonize their tumors of origin, in a process called "tumor self-seeding." Self-seeding of breast cancer, colon cancer, and melanoma tumors in mice is preferentially mediated by aggressive CTC, including those with bone, lung, or brain-metastatic tropism (Kim et al. 2009). Therefore, counting CTC before and after surgery, or therapy, profiling CTC for their surface markers, deciphering their genetic constituents, and administering correct and personalized interventions in the clinic will be one of the future clinical approaches in patient management.

5 Conclusions and future approaches

It was in 1869 that CTC were first detected, using optical microscopy, in the blood of a patient suffering from a metastatic disease. It has taken us more than 140 years to recognize that CTC counts could be used as prognostic indicators of disease progression and survival. Nevertheless, the recent progress in the area of micro- and nanotechnology has led us to create many different types of ex vivo CTC detectors with high yields (90–100%). CTC detection in vivo is a big challenge and can sample a much larger volume of blood compared to patient blood draws. If in vivo methods of monitoring become successful, it can set the stage for capturing CTC at a much earlier stage, thereby enhancing survival. The biological aspects of CTC need to be well delineated in order to understand the EMT process, the timing of CTC release, and the stem cell-like properties of CTC. These insights could galvanize the development of future drugs to block metastases. Micro- and nanotechnology approaches will definitely continue to impact the area of CTC research both in vitro and in vivo. Nanoscale devices will be more suitable in vivo due to their small size, thereby enabling molecular targeting. Nanopatterned surfaces in a micro-fluidic channel can be effective in capturing CTC. Further, analysis of such captured CTC using MEMS-based optical/electronic/magnetic approaches can lead to a handheld system that will enable clinicians not only to rapidly detect cancer cells but also to identify immunohistochemical receptors that might enable the stratification of patients for a specific therapy. The simultaneous detection of CTC and selective killing of CTC in blood vessels could result in minimally invasive treatments for cancer that could result in longer survival and lower cost. Batch fabrication of micro- and nanosensors can take the level of CTC detectors down to a fraction of the cost compared to Veridex ($600 per sample) or MRI and CT scans (>$1,500 per scan). Micro- and nanotechnology, if properly implemented, can lower the cost of CTC detectors, improve sensitivity and specificity, translate the laboratory detection of CTC to clinical settings, and ultimately stratify patients or assess therapeutic outcomes at a rapid pace in a clinical setting. Each of these steps is likely to increase the overall survival of patients.

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