Journal of
Carcinogenesis & Mutagenesis

Tamimi et al., J Carcinog Mutagen 2015, 6:4
DOI: 10.4172/2157-2518.1000229

Review Open Access

Micrometastatic Circulating Tumor cells; A Challenge for an Early Detection and Better Survival Rates

Yahya Tamimi[1*], Ishita Gupta[2], Mansour Al-Moundhri[3] and Ikram Burney[3]

[1]*Department of Biochemistry, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, PC 123, Sultanate of Oman*

[2]*Department of Genetics, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, PC 123, Sultanate of Oman*

[3]*Department of Surgery, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, PC 123, Sultanate of Oman*

[*]**Correspondence author:** Yahya Tamimi, Department of Biochemistry, College of Medicine and Health Sciences, PO Box 35, PC 123 Al Khoud, Sultanate of Oman, Tel: +96824143522; E-mail: yahyatam@squ.edu.om

Rec date: Jun 02, 2015; **Acc date:** Jun 29, 2015; **Pub date:** Jul 02, 2015

Abstract

Micrometastasis is a health burden affecting a large population worldwide, where early stage circulating tumor cells are clinically below the detection limit of the currently used techniques in diagnosis. These cells are considered one of the sources related to the disease spread, usually associated with poor prognosis and resistant to conventional therapies. With the recent advances in technology, various molecular and biological techniques including cytological examination, RT-PCR immunocytochemistry, immuno-magnetic separation and cell-enrichment techniques have emerged to improve the early detection of circulating tumor cells in different carcinomas. However, the sensitivity and specificity of these techniques along with their prognostic influence are still contested. This review aims to discuss the role of key player molecules including cell adhesion molecules, integrins and proteases in promoting micrometastasis and the current techniques used for an early detection of these malignant cells. Understanding mechanisms underlying this invasive process, will pave the way for designing new tools to unravel difficulties associated with early detection of CTCs and will improve therapies.

Keywords: Cancer cells; Micrometastasis; Cell-adhesion molecules; Integrins; Proteases

Introduction

Cancer is defined as a class of diseases characterized by an uncontrolled cell growth with major hallmarks including resistance to apoptosis, alteration of growth factors (GFs), uncontrolled cell-cycle promoting limitless replications, metastasis and angiogenesis [1]. Extensively altered cells do not respond to regulatory stimulus and tend to form lumps leading to malignant tumors, which can either be confined to the related organ or start spreading by invading of the basement membrane and/or formation of new blood vessels. Cancer is very heterogeneous and variability might be seen among different ethnicities and races, although individuals from different regions might also harbor the same tumor [1]. The process of spread from the primary site of the tumor to proximate or distant sites is considered one of the most threatening aspects of cancer [2]. It is the primary cause of treatment failure and cancer-related death. Cancer cells tend to invade secondary sites (i.e., bone, liver, lung, brain) and exfoliate into body cavities, especially the pleural space where they grow in suspension within effusions [3]. Metastatic cells circulate in the bloodstream even after trials to eradicate the primary tumor and remain often undetectable at diagnosis. These circulating tumor cells, suspected to be the origin of metastatic disease, can be easily found in the peripheral blood and bone marrow of cancer patients.

Despite the tremendous efforts and advances in the treatment of cancer, recurrence continues to be intriguing and pose serious challenges to clinical managements. The establishment of this complex process depends essentially on the ability of cancer cells to acquire not only a migratory phenotype but also the capacity to create a secondary niche in a distance. To accomplish this, cancer cells undergo substantial changes through a multistep process, initiated by the dissociation of the malignant cell from the primary site to enter the bloodstream (intravasation) and develop a survival mechanism to the hostile environment before settling on the target tissue or organ (extravasation) and propagate. It is believed that cells engaged in such processes after acquiring an aggressive phenotype, tend to become invasive by migrating through the basement membrane. To promote invasiveness, a complex molecular process involving components such as cell adhesion molecules (CAMs), secreted proteases (metalloproteinases; MMPs), integrins and other specific genes (e.g., Snail, YB-1) act conjointly to allow the loss of cell adhesion and facilitate cell migration [4].

The outcome of such phenotype is the establishment of micrometastasis, a situation where tumor cells remain clinically not easy to detect by conventional methods and develop resistance to therapy. These circulating tumor cells claimed to be the source of the metastatic disease associated with poor prognosis. However, not all affected patients develop metastasis since a fraction of these cells may either die or adopt a dormant state until conditions are favourable to turn active again with an invasive potential. This underlies the biological complexity, not yet fully understood of the metastasis phenomena. Several studies have aimed to characterize the phenotype of these circulating tumor cells and revealed that developing an aggressive phenotype resistant to therapy is likely due to the absence and/or down regulation of key genes such as the proliferation associated antigen Ki67 and the adhesion molecules E-cadherin [4].

This review will shed some light on mechanisms ruling metastasis by focusing on the key molecules involved and the techniques (Table

1) that can be used for an early detection to improve survival rates in an attempt to eradicate cancer.

Techniques used for CTCs Detection	Targeted Material
Cytology	Epithelial cells in blood samples
RT-PCR immunocytochemistry	RNA extracted from epithelial cells
immunomagnetic cell capture	Epithelial cells in blood samples
RT-PCR	RNA extracted from epithelial cells
Quantitative RT-PCR	RNA extracted from epithelial cells

Table 1: Techniques used for CTCs detection.

Role of Cell Adhesion Molecules, Integrin and Proteases

Cell adhesion molecules (CAMs)

Recently, gene expression profiling using microarrays revealed the expression pattern of different genes involved in the cascade of events leading to cancer [5]. Among these, epithelial cell adhesion molecules (Ep-CAM) and E-cadherin were found to be the vital players involved in the complex process of invasion and metastasis [6]. Cadherins are proteins highly dependent on Ca^{2+} ions for their cell-cell adhesion activity and are characterized by five repeated cadherin-specific motifs in their extracellular domain [7]. E-cadherin, a member of the large cadherin superfamily is a trans-membrane glycoprotein that mediates calcium-dependent intercellular adhesion, particularly the epithelial cell-cell adhesion [8,9]. The gene coding for E-cadherin is located on chromosome 16q22.1 and plays a regulatory role in morphogenesis [10]. Moreover, E-cadherin is reported to have a role in carcinogenesis mainly during invasiveness where its expression has been reported to be systematically down regulated [11-13]. E-cadherin loss was reported to be directly associated with invasiveness acquisition and higher tumor stages in prostate [14,15], gastric [16], colorectal [17] and breast [18,19] cancers. Thus, E-cadherin was qualified as a tumor suppressor gene playing a key role in the transition from premalignant lesions to invasive metastatic cancer [20]. Moreover, there is compiling evidence that interaction of E-cadherin with β-catenin plays a crucial role in Wnt signaling pathway involved in carcinogenesis and aggressive phenotype development [21,22].

Contrastively, E-Cadherin is overexpressed in certain cancer cases and not systematically associated with a gradual loss of expression correlating with an increase in stage. Recent studies reported a higher frequency of E-cadherin expression in primary sites of breast cancer as well as in gastric cancer [23,24].

The status of E-Cadherin protein in the determination of the CTC has not been clarified yet. However, in bladder cancer, elevated circulating E-cadherin levels correlated with the disease progression but failed to reach statistical significance, suggesting that soluble E-cadherin levels are not able to predict patients' prognosis. Thus, molecular markers predicting disease progression to discriminate high-risk patients and improve decisions about treatment are still needed [25]. Although the mechanism of promoting cancer progression by the loss of E-cadherin function is not yet well explored, efforts have been deployed to clarify its potential to regulate β-catenin and block the mitogenic signaling through growth factor receptors underlining the complexity of E-cadherin tumor suppressor function [26].

Another subgroup of cadherin superfamily, FAT proteins, a cell adhesion-component of Hippo signaling pathway involved in controlling organ size consists of more than 80 members in mammals, seems to play a crucial role in cancer spread and metastasis [27-29]. Recently, a study involving next-generation sequencing (NGS) in murine oral squamous cell carcinoma identified conservation of human driver pathway alterations in Trp53, MAPK, PI3K, NOTCH, JAK/STAT and FatI-4 [30].

In Drosophila, mutations/deletions causing loss of function of the Fat gene generate hyperplasia of the pupal imaginal disks [31] suggesting that Fat has a suppression effect on tumors. Moreover, loss-of-function of the Fat gene is directly linked to an excessive cell proliferation with normal epithelial organization and differentiation potential [32]. Moreover, Fat4 expression was found to be lost in a large fraction of human breast tumor cell lines and primary tumors. In breast cancer for instance, the loss of Fat4 expression was found to be induced by the promoter methylation[33]. These findings strongly suggest that Fat4 is a potential candidate for a breast tumor suppressor gene [33].

Role of integrins in metastasis

Integrins belong to the family of adhesion receptors and are also involved in extracellular matrix adhesion. In mammals, integrin genes 18a and 8b encode polypeptides that form 24 a,b heterodimer receptors by combination [34,35]. The combined extra-cellular domains consist of large extracellular matrix and cell surface ligands, while the cytoplasmic domains engage actin cytoskeleton via a series of linker proteins [34,35].

Integrins adopt known endocytic pathways, paving the path for the receptors to promote cell migration in either two dimensions due to loss of focal adhesion [36-38] or three dimensions by direct interactions between avb6 integrin and HAX-1 control receptor endocytosis [39]. Following endocytosis, integrins, are arranged in early endosomes to be degraded through a slow process as compared to endocytic inhibition, suggesting its crucial role in the regulation of integrins to be present at the plasma membrane [40-44]. Several studies have shown inhibition of integrins to be involved in adhesion complex formation and migration in 2D [41-43]. Furthermore, trafficking of integrins have been suggested to be involved in regulating invasive migration in 3D [45,46].

Integrins, avb3 and a5b1 tend to bind to similar ligands, however; while both integrins promote migration, they can simultaneously suppress each other by stimulating variant signaling responses [47]. In the absence of fibronectin, phosphorylation of rabaptin-5 by PKD promotes Rab4-dependent avb3 inhibition, thus promoting migration in 2D and invasion into 3D extracellular matrix (ECM) [48,49]. On the other hand, in the presence of fibronectin, invasion is inhibited. This antagonist activity is due to the inhibition and pro-invasive activity of a5b1 which couples with Rab-coupling protein (RECP). Rab is an effector to recruit receptor tyrosine kinases and control their trafficking and signaling to promote invasion into fibronectin-rich ECM [48-52], a condition generally observed in ovarian cancer [53].

Glycans and glycoproteins composing the cellular glycocalyx are also described to be associated to malignancy. In a recent report, glycocalyx was found to aid in the grouping of integrins by channeling active ingredients into adhesions. Clinical experiments from patients

with advanced stages of cancer showed significant expressions of bulky glycoproteins on CTCs, representing the prominent characteristic of tumor cells that can promote metastasis by mechanically altering cell-surface receptor function [54].

In a study conducted in breast cancer patients, bone marrow micrometastasis showed upregulation of ICAM-1 and αvβ2 integrins, suggesting the pro-angiogenic nature of micrometastatic cells and the possibility to design therapeutic strategies [55]. In lung cancer, it has been shown that interactions between the tumor and surrounding ECM is initiated by the formation of thin, actin-rich protrusions which hold integrin β-1 with other proteins to allow cellular-matrix adhesion. These interactions are the result of the formed protrusions that allow cells to trigger signaling cascades such as the FAK pathway involved in adhesion. This leads in turn to ERK phosphorylation and activation allowing proliferation of cancer cells [56].

Although CTCs detection are technically very challenging, requiring very specific and sensitive methods, they remain however an invaluable source of tumor cells and promising biomarkers. Therefore, novel and sophisticated strategies were developed for detecting viable and tissue-specific CTCs using a tropism-enhanced and conditionally replicating reporter adenovirus (CTC-RV). Viral tropism was expanded through capsid-displayed integrin targeting peptides, suggesting the indirect role of integrin to detect viable CTCs with cell specificity and high sensitivity [57].

Role of proteases in metastasis

Proteases are enzymes that specifically degrade and destroy the ECM and basement membrane along with remodeling of the tissue leading to invasion and metastasis [58]. This section will discuss the various classes of proteases involved in tumor invasion and metastasis including relevant candidates such as cysteine, aspartate, threonine, serine and matrix metalloprotesases [59].

Cysteine proteases are mainly found in the lysomes (e.g., cathepsins B, L, H and S) or in the cytosol such as calpains, involved in the breakdown of both, intra and extracellular matrix proteins [60]. This digestion property promotes the ability to invade the surrounding tissues, blood and lymph nodes and metastasize to distant tissues [61]. Cathepsins have been used as markers for diagnosis in breast [62], colon [63], tongue [64]and pancreatic cancers [65]. Furthermore, cathepsins play an important role in angiogenesis regulation and therefore, actively involved in tumor progression [60]. Cathepsin B was the first identified lysosomal protease to be linked to breast carcinoma [66]. It has the capability to degrade and remodel the connective tissue as well as the basement membrane by secreting lysosomes. This is considered as an important step in invasion and metastasis [67]. Interestingly, upregulated levels of cathepsins H, L and D have also been reported in various cancers. For instance, cathepsin L2 (CTSL2) was shown to be upregulated in breast, lung, endometrial [68], gastric, colon, head and neck, skin cancer and gliomas [69].

The aspartate protease (cathepsin-D) is localized in the lysosome and is highly expressed and secreted in large amounts by human epithelial breast cancer cells and has been developed as a marker of poor prognosis in breast cancer [70].

Proteasomes or threonine proteases are involved in polyubiquitinization, a complex process through which they degrade and eliminate cellular proteins. Mutated proteasome-dependent proteolysis has found to be linked with the onset of certain malignancies [59].

On the other hand serine proteases are associated with cell growth and differentiation. Urokinase-type plasminogen activators are shown to be involved with invasion and metastasis, while a type II transmembrane protease, matriptase is associated with the regulation of angiogenesis, ECM degradation and tumor progression [71,72]. One of the known serine proteases, trypsin was reported to have a role in colorectal cancer and promotes cellular proliferation, invasion and metastasis [73]. Although, trypsin overexpression in colorectal cancer is associated with poor prognosis and poor survival, the underlying mechanism ruling trypsin involvement in tumor progression is still unclear. Trypsin and protease-activated receptor-2 (PAR-2) conjointly promote cellular growth, invasion and metastasis [74]. Furthermore, it has been shown that trypsin act along with matrix metalloproteinases-2,-7 and -9 (discussed below) to cause invasion and metastasis [75].

Matrix metalloproteinases (MMPs) are members of the proteases family that play a crucial role in the cleavage of cell surface receptors, and the regulation of ligands such as FAS and chemokine/cytokine inactivation affecting cell growth, migration, angiogenesis and apoptosis [59]. MMPs have the potential to degrade the ECM and are responsible for the conversion of adenomas to carcinomas in addition to the initiation of invasion and metastasis [76]. While MMPs-1,-2,-3,-7,-9,-12 and -13 are involved with tumor progression, MMPs-2 and-9, known as gelatinases are associated with tumor invasion and metastasis in several tumors. Interestingly, the NF-κB upregulation was shown to be associated with the over-expression of MMP-9, resulting in ECM and cell adhesion degradation, promoting invasion and micrometastasize [77]. Interestingly, the MMP-2 expression profile closely correlated with micrometastasis and invasiveness, and therefore emerged as a potential progression marker [78].

Another class of proteinases, ADAMTS, belongs to the family of secreted, matrix-associated enzymes that have a variety of functions in regulating tissue organization and vascular homeostasis. At least 19 of them have been found to play a role in tumor promoting or inhibiting in humans. While, a study identified an elevated ADAMTS expression associated with worst clinical outcome in mammary carcinoma [79], a recent study in breast cancer patients, discovered elevated levels of ADAMTS to be associated with better outcomes, indicating a controversial role. However, it has been noted that various members of the ADAMTS family inhibit cancer, as they are generally silenced or corrupted in tumor cells. A study conducted, using both wild-type and MMP-deficient mutant ADAMTS-15 on breast cancer cell lines, revealed no effect on cellular proliferation and cell death. Furthermore, the study described that the wild-type hampers angiogenesis. Interestingly, forms, affected metastasis and the effect being subjected to the tissue environment of the target organ [80].

Moreover, other relevant markers contributing to cancer spread were also identified. Among these, E-selectin ligands expressed by circulating tumor cells [81] showed convincing evidence in promoting metastasis in several cancers including head and neck and breast cancer [82-85]. Selectin ligand E, L and P were found to be expressed on colon cancer cells, while E-selectin ligand was found on prostate and breast cancer cells [81,86,87]. Though the understanding of these markers is growing, it is therefore important to consider their biochemical and biophysical utility to track CTCs in transit.

"Anoikis" an Alternative form of Programmed Cell Death

In order to obstruct the way for any metastasis progress, following cell-cell contact and extracellular matrix loss, cells enter another form of cell programmed death called "anoikis" [88]. The latency time of recurrence recorded in some patients between the initial attempted therapy to eradicate the primary tumor and relapse is attributed to this anoikis phase. It is a process involved in homeostasis regulation, and plays a crucial role in wound healing and tissue remodeling during development [89,90]. Resistance to anoikis occurs through a complex process including the activation of oncogenes such as PI3K and Akt and/or the loss of key tumor suppressor genes [91-94]. Therefore, in order to survive while circulating after detachment from the primary tumor and prior to metastasis, cells develop sophisticated mechanisms to resist anoikis. Such resistance has been observed in several cancers and is thought to significantly contribute to the aggressive phenotype as well as the survival of the invading cells and metastasis [91,95]. Thus, understanding mechanisms underlying the resistance to anoikis would provide a standard way to investigate micrometastasis regulation and help tailoring novel therapies to eradicate cancer.

Emergent tools for micrometastasis detection

Interest in circulating micrometastic cancer cells had already started to develop in the 19th century (1869) when it was noticed that cells resembling primary-tumor-cells were found in the blood of some patients after death [96]. Thereafter, different new techniques emerged to improve the detection of circulating tumor cells in various types of carcinomas using different cytological methods [97,98]. With the advent of immunocytochemistry, cytological examination of blood samples became a routine procedure to detect circulating tumor cells in blood with much higher sensitivity when compared to conventional techniques [99-101]. Indeed, these assays were able to identify spiked tumor cells in 6 to 15 ml of blood samples containing between 10,000 to 100,000 mononuclear cells[102], suggesting that techniques such as immunocytochemistry provide an additional value in terms of prognostics [103,104]. However, due to several factors including loss of antigen expression in poorly differentiated tumors, this technique was not used as a routine procedure in cancer staging protocols [105-107].

The polymerase chain reaction (PCR), a highly sensitive nucleic acid-based technique, emerged to revolutionize the conventional detection methodology used to identify circulating tumor cells in different cancers such as leukaemias, lymphoma, and other solid tumors [108-110]. The advent of PCR technique made an enormous impact upon nucleic acid analysis, allowing the amplification of specific DNA fragments flanked by designed oligonucleotides, using repeated cycles including denaturation, annealing and elongation steps [111]. PCR was revealed to be a very sensitive tool allowing the detection of one malignant cell among more than 100 normal cells [112-115]. Tumors with characterized molecular abnormalities such as leukemia were among the target for PCR while for solid tumors, other strategies including targeting tumor markers were developed [116,117]. These included the amplification of immunoglobulin heavy chain gene t(14;18)(q32;q21)] or specific oncogene mutations that can be used to identify malignant cells [116,118]. Interestingly, the combination of PCR with other techniques such as reverse transcriptase and immunocytochemistry improved the sensitivity and specificity allowing the identification and the enrichment of malignant circulating tumor cells [113,115,119]. The choice of the amplified target determined by specific characteristics of the malignant cells seems to be the limiting factor to identify circulating tumor cells using both mRNA and genomic DNA materials. Therefore, specific aberrations (mutations, amplifications) present within genomic DNA of malignant cells are potential targets to specifically discriminate and isolate circulating cancer cells. The big challenge this process poses consists of identifying cancer cells circulating amongst millions of leukocytes and erythrocytes and discriminating them from epithelial noncancerous cells in a given volume of blood. Due to certain PCR limitations such as contaminations, other approaches emerged for better detection and characterization of circulating tumor cells at the molecular level. Crossing over region found on certain chromosomes [i.e., Philadelphia chromosome, t(9;22)(q34;q11 and bcl2] and Immuno-magnetic separation technology, a technique where the specimen is incubated with magnetic beads coated with antibodies directed against specific antigens exclusively expressed by cancer cells were used as a mean to improve selection and enrichment [120-123]. For instance, the anti-epithelial antibody Ber-EP4 directed against carcinomas was used to enrich cancer cells disseminated in blood stream, while the anti-leukocyte antibody CD45 was used for depletion of mononuclear cells using a magnet [124]. Moreover, several bladder biomarkers have been investigated for their screening potential and higher sensitivity to detect urothelial malignant growth [125,126].

The enriched cells can be analyzed using Immunocytochemistry providing access to more information concerning the assessment of tumor specific proliferation and progression markers, as well as quantification of tumor cells which is a great help to monitor the impact of targeted therapy. This would improve the stratification of patients with solid tumors and better elucidate the dynamic process of metastases.

Quantification of CTCs may be used as a potential prognostic marker that could guide treatment decisions and/or monitor the response to treatment. In a phase-II randomized trial of advanced breast cancer, the detection of CTCs predicted an early metastatic relapse following neoadjuvant therapy [127,128]. In prostate cancer, a concordance between circulating prostate cancer cells in the blood and the dissemination of cancer cells to distant organs (e.g., bone) was observed for all Gleason scores. For bone marrow biopsies however, this concordance was observed only for high grade tumors up to Gleason score 9 [129].

Since the introduction of RT-PCR technique, mRNA is increasingly used as a target for the detection of tumor cells, allowing the detection of translocations and other rearrangements which occur within introns [119]. To be more specific and have less background related to unspecific priming, magnetic beads can also be coated with oligo (dT) to specifically isolate mRNA from the total RNA extracted from the enriched population of cells. In breast cancer for instance, mRNA from both EGF-R and cytokeratin 19 displayed a profile of highly specific and sensitive biomarkers with the potential to discriminate and detect metastasizing breast cancer cells among normal peripheral blood mononuclear cells [130]. To selectively amplify cDNA produced from mRNA, it is capital to avoid genomic DNA contamination, which can be a drawback in few cases even after RNAse-free DNAse treatment. Therefore, primers design should span an intron resulting in the amplification of different products with genomic DNA contaminate samples that generate bigger size products when compared to spliced mRNA [119].

Undetected micrometastastic circulating cells lead inevitably to relapse and therefore, the identification of patients with an early-stage

cancer may have a substantial impact not only on prognosis but also on the choice for the therapy used [131]. Thus, the necessity to improve the detection and identification of CTCs in blood to optimize management of cancer patients is important. Methods such as cytology and RT-PCR enable to enrich micrometastatic circulating cells from blood [132], and may aid in the early detection of cancer when tumors are still confined and there is still more hope to complete cure [133].

In breast cancer, the use of antibodies directed against breast cancer epithelium was able to detect CTCs in 95% and 32% of breast cancer patients before and after surgery respectively [134]. These promising results was the primary motivation to design studies to detect circulating cancer cells using sensitive and specific molecular techniques such as immunomagnetic cell capture coupled with quantitative RT-PCR (qRT-PCR) [135].These methods have been proven to be extremely sensitive, being able to detect only four cells per 10 ml of blood [136]. It has been shown that circulating breast cancer cells are released into the blood at an early stage of the disease and a substantial number of patients at the time of diagnosis have already circulating micrometastatic cells [134].

Cell enrichment technique along with novel emerging molecular technologies provide the right tools to isolate and characterize circulating tumor cells and potentially provide important diagnostic and prognostic tests [132,137,138]. In a prospective study on a large cohort of metastatic breast cancer patients, the significance of prognosis associated with circulating tumor cell levels showed that patients with higher circulating tumor cells (5 cells per 10 ml of blood) had a shorter progression-free survival and shorter overall survival ($P<0.001$) [139]. Although current research to improve circulating tumor cells capture is often satisfactory, it remains however ambiguous for some challenges such as sensitivity, specificity and interpretation [140]. Therefore, second generation technology essentially based on advanced technology allowing for counting, capturing, and characterizing tumor cells found in a patient's blood, is now available in reputed research institutes. Preliminary results are encouraging with the potential to personalize these applications to cancer therapy and the possibility to change treatment regimen if the number of circulating tumor cells are not reduced after the first treatment [140]. Yet, more effort should be deployed to improve specificity and reproducibility of circulating tumor cells assays.

Clinical Applications of Circulating Tumor Cells

Molecular characterizations of CTCs have the potential to play polyvalent roles in the pathogenesis process including being used as biomarkers surrogate for overall survival prognosis, staging, biomarker discovery and personalizing treatment by serving as 'liquid-biopsies' [141]. In breast cancer for instance, CTC has been explored successfully as a surrogate for HER2 expression/amplification [142] and alterations in CTC count may aid in indicating sensitivity or resistance to various cancer treatments [141]. In prostate cancer, prostate-specific antigen (PSA) levels, weakly associated with better survival is not sufficient to guide treatment in the first trimester [143]; in few cases of highly advanced and androgen receptor driven (AR-driven) stages, PSA fails to be reliable. Hence, bone scans are required every 6 weeks to avoid any relapse in response to the treatment. To overcome these limitations, Veridcex Cell Search System, an FDA-cleared assay for the enumeration of CTCs [141,144], was approved based upon several studies carried out on breast [145], prostate [146] and colorectal cancers [147, 148]. This system is based on the principle of automated immunomagnetic selection of EpCAM and creatinine kinase positive cells accompanied by anti-CD45 antibodies to eliminate leukocytes and nuclear staining (DAPI) [141]. DAPI stains positive for cytokeratinins and negative for CD45 [149]. The first study using this technique on survival was carried out in 2008. Recently, phase III studies in men with metastatic castration resistant prostate cancer (mCRPC) undergoing treatment with either docetaxel [150], or docetaxel and prednisone with or without lenalidomide were carried out. Interestingly, the prognostic value of CTCs using Cell-Search-Assay [151] was confirmed with a better prognostic value and over-survival rate when change from >5CTCs to <5CTCs counted cells [150,151].

To further elaborate the use of CTC enumeration for better prognosis and management of patients, phase III studies in breast cancer were carried out [142] and evaluated the role of CTCs in guided hormonal therapy. In metastatic patients, CTC count tends to change during treatment with anti-HER2 based on CTC detection. The data outcome revealed that CTC testing improved the prognostic and the overall survival rate of patients with metastatic breast cancer [142].

While, the major challenge for CTC enumeration is the tumor heterogeneity of the CTC enriched cells, algorithms for wholly performing automated counting of CTCs were optimized [152]. Furthermore, several innovative platforms, marker-independent and qualified to optimize the isolation of CTCs is also under active investigation [153-155] to provide better prognosis services and improve the overall survival rates.

Conclusion

The complete understanding of how cancer cells transit the border line from primary stage to disseminated tumor cells and how these cells can release mutated DNA to the interstitial area remains unclear. During the multistep process toward the establishment of new metastatic niche, circulating cells undergo the influence of a plethora of biochemical and biophysical stresses conducting cells towards an aggressive phenotype. The consequences of metastasis are thought to be the main cause of cancer related mortality rather than primary tumors. Recurrence that usually follows first treatment is thought to stem from circulating tumor cells already existing at the time of the operation [156,157]. Thus, the detection procedure of circulating tumor cells in peripheral blood becomes very popular in predicting relapse and metastasis and can also contribute to better diagnosis. Several studies have reported the detection of circulating cancer cells using the available molecular biology tools such as magnet bead cell-capture and RT-PCR [158-160].

On the other hand, detection of mutated circulating free DNA is an emerging promising technique to not only be a surrogate for tumor tissue DNA but also a tool for metastasis prediction and diagnosis [161-163]. The outcome of these research efforts is to develop these noninvasive markers in order to achieve effective and better-tailored anticancer treatments and improve life expectancy for affected individuals.

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