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

Yahya Tamimi1*, Ishita Gupta2, Mansour Al-Moundhri2 and Ikram Burney3
1Department of Biochemistry, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, PC 123, Sultanate of Oman
2Department of Genetics, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, PC 123, Sultanate of Oman
3Department of Surgery, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, PC 123, Sultanate of Oman

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 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 compelling 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 Fat4 [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 raptabin-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 αvβ3 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 β1 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 metalloproteases [59].

Cysteine proteases are mainly found in the lysosomes (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 polyubiquitination, 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, matripase 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-kB 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 micrometastatic 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 leukaemias 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 (14;18) 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 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 unspecified 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 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 micrometastatic 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, Veridex 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 agressive 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.

References

1. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646-674.
2. Perry M (2011) Approach to the patient with cancer. In: Goldman L, Schafer AI (eds.) Cecil Medicine (24thedn.) Saunders Elsevier, Philadelphia, Pa.
3. Carmichael A, Sami AS, Dixon JM (2003) Breast cancer risk among the survivors of atomic bomb and patients exposed to therapeutic ionising radiation. Eur J Surg Oncol 29: 475-479.

4. Li LT1, Jiang G, Chen Q, Zheng JN (2015) Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep 12: 1566-1572.

5. Fehrmann RS, Li XY, van der Zee AG, de Jong S, Te Meerman GJ, et al. (2007) Profiling studies in ovarian cancer: a review. Oncologist 12: 960-966.

6. Heinzlmann-Schwarz VA, Gardiner-Garden M, Henshall SM, Scurry J, Scully RA, et al. (2004) Overexpression of the cell adhesion molecules DDR1, Claudin 3, and Ep-CAM in metastatic ovarian epithelium and ovarian cancer. Clin Cancer Res 10: 4427-4436.

7. Shapiro L, Fannon AM, Kwong PD, Thompson A, Lehmann MS, et al. (1995) Structural basis of cell-cell adhesion by cadherins. Nature 374: 327-337.

8. Deman JJ, Van Larebeke NA, Bruynneel EA, Bracke ME, Vermeulen SJ, et al. (1995) Removal of sialic acid from the surface of human MCF-7 mammary cancer cells abolishes E-cadherin-dependent cell-cell adhesion in an aggregation assay. In Vitro Cell Dev Biol Anim 31: 633-639.

9. Gumbiner BM (2000) Regulation of cadherin adhesive activity. J Cell Biol 148: 399-404.

10. Day ML, Zhao X, Vallorosi CJ, Putzi M, Powell CT, et al. (1999) E-cadherin mediates aggregation-dependent survival of prostate and mammary epithelial cells through the retinoblastoma cell cycle control pathway. J Biol Chem 274: 9656-9664.

11. Chen WC, Obirin B (1991) Cell-cell contacts mediated by E-cadherin (involucrin) restrict invasive behavior of L-cells. J Cell Biol 114: 319-327.

12. Fri xen UH, Behrens J, Sachs M, Eberle G, Voss B, et al. (1991) E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol 113: 173-185.

13. Piercell WE, Woodard AS, Morrow JS, Rimm D, Fearon ER (1995) Frequent alterations in E-cadherin and alpha- and beta-catenin expression in human breast cancer cell lines. Oncogene 11: 1319-1326.

14. Umbas R, Isaacs WB, Bringuique PP, Schaafsa ME, Karthaus HF, et al. (1994) Decreased E-cadherin expression is associated with poor prognosis in patients with prostate cancer. Cancer Res 54: 3929-3933.

15. Umbas R, Isaacs WB, Bringuique PP, Xue Y, Debruyne FM, et al. (1997) Relation between aberrant alpha-catenin expression and loss of E-cadherin function in prostate cancer. Int J Cancer 74: 377-374.

16. Mayer B, Johnson JP, Leitl F, Jauch KW, Heiss MM, et al. (1993) E-cadherin expression in primary and metastatic gastric cancer: down-regulation correlates with cellular dedifferentiation and glandular disintegration. Cancer Res 53: 1690-1695.

17. Dorudi S, Hanby AM, Poulson R, Northover J, Hart IR (1995) Level of expression of E-cadherin mRNA in colorectal cancer correlates with clinical outcome. Br J Cancer 71: 614-616.

18. Oka H, Shiozaki H, Kobayashi K, Inoue M, Tahara H, et al. (1993) Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. Cancer Res 53: 1696-1701.

19. Rashbridge SA, Gillette CE, Sampson SA, Walsh FS, Mills RR (1993) Epithelial (E-) and placent (P-) cadherin cell adhesion molecule expression in breast carcinoma. J Pathol 169: 245-250.

20. PeA1ina-Slaus N (2003) Tumor suppressor gene E-cadherin and its role in normal and malignant cells. Cancer Cell Int 3: 17.

21. DiMeo TA, Anderson K, Phadke P, Fan C, Perou CM, et al. (2009) A novel lung metastasis signature links Wnt signaling with cancer cell self-renewal and epithelial-mesenchymal transition in basal-like breast cancer. Cancer Res 69: 5364-5373.

22. Huang D, Du X (2008) Crosstalk between tumor cells and microenvironment via Wnt pathway in colorectal cancer dissemination. World J Gastroenterol 14: 1823-1827.

23. Cho SJ, Kook MC, Lee JH, Shin YJ, Park J, et al. (2015) Peroxisome proliferator-activated receptor γ upregulates galectin-9 and predicts prognosis in intestinal-type gastric cancer. Int J Cancer 136: 810-820.

24. Fulva V, Rudico L, Balica AR, Cimpian AM, Saptefrati L, et al. (2015) Differential expression of e-cadherin in primary breast cancer and corresponding lymph node metastases. Anticancer Res 35: 759-765.

25. Szaras T, Hoffmann F, Becker M, Schenck M, Vorm Dorr F, et al. (2011) [Plasma E-cadherin levels in urinary bladder cancer: does it improve risk stratification?]. Urolologie A 96: 64-70.

26. Jeanes A, Gottardi CJ, Yap AS (2008) Cadherins and cancer: how does cadherin dysfunction promote tumor progression? Oncogene 27: 6920-6929.

27. Steinberg MS, McNutt PM (1999) Cadherins and their connections: adhesion junctions have broader functions. Curr Opin Cell Biol 11: 554-560.

28. Takeichi M (1991) Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251: 1451-1455.

29. Yagi T, Takeichi M (2000) Cadherin superfamily genes: functions, genomic organization, and neurologic diversity. Genes Dev 14: 1169-1180.

30. Onken MD, Winklel AE, Kanchi K-L, Chalivendra V, Law JH, et al. (2014) A Surprising Cross-Species Conservation in the Genomic Landscape of Mouse and Human Oral Cancer Identifies a Transcriptional Signature Predicting Metastatic Disease. Clinical Cancer Research 20: 2873-2884.

31. Mahoney PA, Weber U, Onofrechuk P, Biessmann H, Bryant P, et al. (1991) The fat tumor suppressor gene in Drosophila encodes a novel member of the cadherin gene superfamily. Cell 67: 853-868.

32. Garcia F, Guerra D, Pezzoli MC, López-Varela A, Cavicchi S, et al. (2000) Cell behaviour of Drosophila fat cadherin mutations in wing development. Mech Dev 94: 95-109.

33. Qi C, Zhu YH, Yu L, Zhu YJ (2009) Identification of Fat4 as a candidate tumor suppressor gene in breast cancers. Int J Cancer 124: 793-798.

34. Hynes RO (2002) Integins: bidirectional, allosteric signaling machines. Cell 110: 673-687.

35. Wolfenson H, Lavelin I, Geiger B (2013) Dynamic regulation of the structure and functions of integrin adhesions. Dev Cell 24: 447-458.

36. Chao WT, Kunz J (2009) Focal adhesion disassembly requires clathrin-dependent endocytosis of integrins. FEBS Lett 583: 1337-1343.

37. Ezraty JJ, Bertaux C, Marcatonio EE, Gundersen GG (2009) Clathrin mediates integrin endocytosis for focal adhesion disassembly in migrating cells. J Cell Biol 187: 733-747.

38. Nishiuma T, Kaibuchi K (2007) Numb controls integrin endocytosis for directional cell migration with apKCa and PAR-3. Dev Cell 13: 15-28.

39. Ramsay AG, Keppler MD, Jazayeri M, Thomas GJ, Parsons M, et al. (2007) H51-associated protein X-1 regulates carcinoma cell migration and invasion via clathrin-mediated endocytosis of integrin alphabeta6. Cancer Res 67: 5275-5284.

40. Röttcher BT, Breitling A, Meves A, Meyer H, Widmaier M, et al. (2012) Sorting nexin 17 prevents lysosomal degradation of β integrins by binding to the β-integrin tail. Nat Cell Biol 14: 584-592.

41. Bridgewater RE, Norman JC, Caswell PT (2012) Integrin trafficking at a glance. J Cell Sci 125: 3695-3701.

42. Chen DY, Li MY, Wu SY, Lin YL, Tsai SP, et al. (2012) The Bro1-domain-containing protein Myopic/HDFTP coordinates with Rab4 to regulate cell adhesion and migration. J Cell Sci 125: 4841-4852.

43. Krondija D, Münzberg C, Maass U, Hafner M, Adler G, et al. (2012) The phosphatase of regenerating liver 3 (PRL-3) promotes cell migration through Arf-activity-dependent stimulation of integrin α5 recycling. J Cell Sci 125: 3883-3892.

44. Steinberg F, Heesom KJ, Bass MD, Cullen PJ (2012) SXN17 protects integrins from degradation by sorting between lysosomal and recycling pathways. J Cell Biol 197: 219-230.

45. Caswell PT, Vadrevu S, Norman JC (2009) Integrins: masters and slaves of endocytic transport. Nat Rev Mol Cell Biol 10: 843-853.
Citation: Tamimi Y, Gupta I, Al-Moundhri M, Burney I (2015) Micrometastatic Circulating Tumor cells; A Challenge for an Early Detection and Better Survival Rates. J Carcinog Mutagen 6: 229. doi:10.4172/2157-2518.1000229

98. Murray NP, Reyes E, Tapia P, Badinez L, Orellana N, et al. (2012) Redefining micrometastasis in prostate cancer - a comparison of circulating prostate cells, bone marrow disseminated tumor cells and circulating tumor cell detection predicts early metastatic relapse after neoadjuvant chemotherapy in large operable and locally advanced breast cancer. Oncotarget 8: 736-740.

99. Pelkey TJ, Frierson HF Jr, Bruns DE (1996) Molecular and immunological detection of circulating tumor cells and micrometastases from solid tumors. Clin Chem 42: 1369-1381.

100. Thomas P, Battifora H (1987) Keratins versus epithelial membrane antigen in tumor diagnosis: an immunohistochemical comparison of five monoclonal antibodies. Hum Pathol 18: 728-734.

101. Cave H, Guidal C, Rohrlich P, Delfau MH, Broyart A, et al. (1994) Prospective monitoring and quantification of residual blasts in childhood acute lymphoblastic leukemia by polymerase chain reaction study of delta and gamma T-cell receptor genes. Blood 83: 1892-1902.

102. Komeda T, Fukuda Y, Sando T, Kita R, Furutaka M, et al. (1995) Sensitive detection of circulating hepatocellular carcinoma cells in peripheral venous blood. Cancer 75: 2214-2219.

103. Miyajima Y, Kato K, Numata S, Kudo K, Horibe K (1995) Detection of neuroblastoma cells in bone marrow and peripheral blood at diagnosis by the reverse transcriptase-polymerase chain reaction for tyrosine hydroxylase mRNA. Cancer 75: 2275-2276.

104. Saiki RK, Bugawan TL, Horn GT, Mullis KB, Erlich HA (1988) Analysis of enzymatically amplified beta-globin and HLA-DQA alpha DNA with allele-specific oligonucleotide probes. Nature 324: 163-166.

105. Cross NC, Feng L, Chase A, Bungey J, Hughes TP, et al. (1993) Competitive polymerase chain reaction to estimate the number of BCR-ABL transcripts in chronic myeloid leukemia patients after bone marrow transplantation. Blood 82: 1292-1296.

106. Datta YH, Adams PT, Drobyski WR, Ethier SP, Terry VH, et al. (1994) Sensitive detection of occult breast cancer by the reverse-transcriptase polymerase chain reaction. J Clin Oncol 12: 475-482.

107. Mattano LA Jr, Moss TJ, Emerson SG (1992) Sensitive detection of rare circulating neuroblastoma cells by the reverse transcriptase-polymerase chain reaction. Cancer Res 52: 4701-4705.

108. Negrin RS, Pesando J (1994) Detection of tumor cells in purged bone marrow and peripheral-blood mononuclear cells by polymerase chain reaction amplification of bcl-2 translocations. J Clin Oncol 12: 1021-1027.

109. Burchill SA, Bradbury FM, Smith B, Lewis JJ, Selby P (1994) Neuroblastoma cell detection by reverse transcriptase-polymerase chain reaction (RT-PCR) for tyrosine hydroxylase mRNA. Int J Cancer 57: 671-675.

110. Smith B, Selby P, Southgate J, Pittman K, Bradley C, et al. (1991) Detection of melanoma cells in peripheral blood by means of reverse transcriptase and polymerase chain reaction. Lancet 338: 1227-1229.

111. Akasaka T, Akasaka H, Yonetani O, Ohno H, Yamabe H, et al. (1998) Refinement of the BCL2/immunoglobulin heavy chain fusion gene in t(14;18)(q32q21) by polymerase chain reaction amplification for long targets. Genes Chromosomes Cancer 21: 17-29.

112. Veres V, Gibbs RA, Scherer SE, Caskey CT (1987) The molecular basis of the sparse fur mouse mutation. Science 237: 415-417.

113. Benez A, Geiselhart A, Handgertinger R, Schiebel U, Fierbeck G (1999) Detection of circulating melanoma cells by immunomagnetic cell sorting. J Clin Lab Anal 13: 229-233.

114. Ghoossein RA, Bhattacharya S, Rosai J (1999) Molecular detection of micrometastases and circulating tumor cells in solid tumors. Clin Cancer Res 5: 1950-1960.

115. Martin VM, Siewert C, Scharl A, Harms T, Heinze R, et al. (1998) Immunomagnetic enrichment of disseminated epithelial tumor cells from peripheral blood by MACS. Exp Hematol 26: 252-264.

116. Racila E, Euhus D, Weiss AJ, Rao C, McConnell J, et al. (1998) Detection and characterization of carcinoma cells in the blood. Proc Natl Acad Sci USA 95: 4589-4594.

117. Zigeuner RE, Riesenberg R, Pohla H, Hofstetter A, Oberneder R (2003) Isolation of circulating cancer cells from whole blood by immunomagnetic cell enrichment and unlabelled immunocytochemistry in vitro. J Urol 169: 701-705.

118. Frantzi M, Makridakis M, Vlahou A (2012) Biomarkers for bladder cancer aggressiveness. Curr Opin Urol 22: 390-396.

119. Ru Y, Dancik GM, Theodorescu D (2011) Biomarkers for prognosis and treatment selection in advanced bladder cancer patients. Curr Opin Urol 21: 420-427.

120. Pierga JY, Bidard FC, Mathiot C, Brain E, Delaloge S, et al. (2008) Circulating tumor cell detection predicts early metastatic relapse after neoadjuvant chemotherapy in large operable and locally advanced breast cancer in a phase II randomized trial. Clin Cancer Res 14: 7004-7010.

121. Bidard F-C, Peeters DJ, Fehm T, Nolé F, Gisbert-Criado R, et al. (2014) Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data. The Lancet Oncology 15: 406-414.
for biochemical failure after radical prostatectomy. Int J Mol Med 30: 896-904.

130. Hildebrandt M, Mapara MY, Korner IJ, Bargou RC, Moldenhauer G, et al. (1997) Reverse transcriptase-polymerase chain reaction (RT-PCR)-controlled immunomagnetic purging of breast cancer cells using the magnetic cell separation (MACS) system: a sensitive method for monitoring purging efficiency. Exp Hematol 25: 57-65.

131. Pantel K, Cote RJ, Fodstad O (1999) Detection and clinical importance of micrometastatic disease. J Natl Cancer Inst 91: 1113-1124.

132. Gilbey AM, Burnett D, Coleman RE, Helen I (2004) The detection of circulating breast cancer cells in blood. J Clin Pathol 57: 903-911.

133. Burchill SA, Selby PJ (2000) Molecular detection of low-level disease in patients with cancer. J Pathol 196: 6-14.

134. Krag DN, Ashikaga T, Moss TJ, Kusminsky RE, Feldman S, et al. (1999) Breast Cancer Cells in the Blood: A Pilot Study. Breast 5: 354-358.

135. Yu M, Stott S, Toner M, Maheswaran S, Haber DA (2011) Circulating tumor cells: approaches to isolation and characterization. J Cell Biol 192: 373-382.

136. Castells A, Boix L, Bessa X, Gargallo L, Piqué JM (1998) Detection of colonic cells in peripheral blood of colorectal cancer patients by means of reverse transcriptase and polymerase chain reaction. Br J Cancer 78: 1368-1372.

137. Jiang WG, Martin TA, Mansel RE (2002) Molecular detection of metastasis in breast cancer. Crit Rev Oncol Hematol 43: 13-31.

138. Muller V, Stahmann N, Riethdorf S, Rau T, Zabel T, et al. (2005) Circulating tumor cells in breast cancer: correlation to bone marrow micrometastases, heterogeneous response to systemic therapy and low proliferative activity. Clin Cancer Res 11: 3678-3685.

139. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, et al. (2004) Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 351: 781-791.

140. Gorges N, Rak J, Jabado N (2010) New technologies for the detection of circulating tumour cells. Br Med Bull 94: 49-64.

141. Friedlander TW, Fong L (2014) The end of the beginning: circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. J Clin Oncol 14: 5.

142. Slanted spiral microfluidics for the ultra-fast, label-free isolation of tumor cells. Lab Chip 14: 128-137.

143. Berthold D, Pond GR, Roessner M, de Wit R, Eisenberger M, et al. (2008) Treatment of hormone-refractory prostate cancer with docetaxel or mitoxantrone: relationships between prostate-specific antigen, pain, and quality of life response and survival in the TAX-327 study. Clin Cancer Res 14: 5.

144. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, et al. (2004) Noninvasive detection of EGFR T790M in gefitinib or erlotinib resistant prostate cancer (mCRPC): The MAINSAIL trial.

145. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, et al. (2004) Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 351: 781-791.

146. de Bono J, Scher HI, Montgomery BB, Parker C, Miller MC, et al. (2008) Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res 14: 7.

147. Lorente D, Mateo J, de Bono JS (2014) Molecular characterization and clinical utility of circulating tumor cells in the treatment of prostate cancer. Am Soc Clin Oncol Educ Book.

148. Colen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, et al. (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: 3213-3221.