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Circulating Tumor Cells (CTCs): An Overview of Current Techniques and Its Potential Clinical Implications

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Abstract

One of the most hopeful developments in cancer field, has been the advent of circulating tumor cells (CTCs) which acts as an important prognostic biomarker of the metastasis. CTCs can be used for patient diagnosis and prognosis, as well as subsequent monitoring of treatment efficacy in cancer patients. Additionally, CTCs can be identified early in the progression of cancer which can be used for initial cancer detection and for anti-cancer treatment. Currently, CTC enumeration is an accepted prognostic indicator for breast, prostate, and colorectal cancer. But various study are going on other cancers besides these three to validate the role of CTCs in diagnostic and prognostic value of metastasis. In recent years, the focus of the researchers has shifted from CTCs enumeration to CTC enrichment and detection which holds great potential for predicting prognosis of cancers. Various detection technologies and devices have been developed to enumerate and characterize CTCs. Most of those approaches are based on the positive enrichment strategy and immune-cytological techniques. Here in this review, we focuses on the various techniques available for the enrichment and detection of CTCs as well as its potential applications in management of various tumors.

Keywords: Circulating tumor cells; CTCs; Cancer prognosis; CTC enrichment; Prognostic biomarker; Epithelial-mesenchymal transition.

Introduction

Circulating tumor cells (CTCs) are defined as tumor cells which are circulating in the peripheral blood of patients, shed from either the primary tumors or its metastases. Subgroups of cancer cells enter the blood circulation after detaching from the primary tumor, travel to a distant part in the body where they can implant themselves and give rise to metastatic new tumor mass. Due to these metastasis, function of an organ (i.e. the lung, the brain, the liver) got hampered which ultimately lead to death of the patients.[1] These circulating tumor cells can be shed at early stages of the disease. In fact, in 30–40% of early cancer patients, which thought to be localised, may be presenting like metastatic tumor due to this early shedding of circulating tumor cells.[2] Therefore, CTCs detection and analysis may be very important for the diagnosis and treatment of cancer patients. But, this early shedding of tumor cells in circulation is usually not detected by high-resolution imaging technologies. Over the past decades, there has been a lot of research has been done for developing new techniques to identify CTCs and researchers are more focussed on i) determining patient prognosis, ii) identifying tumor recurrence, iii) therapeutic responses and iv) understanding mechanism of tumor progression and metastatic disease with the

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help of CTCs. Here, we review the various available methods for the enrichment and detection of CTCs, as well as its potential clinical applications.[3]

Brief historical overview on circulating tumor cells (CTCs)

Circulating tumor cells (CTCs) were first identified in 1869 by Thomas Ashworth in blood of patient who died with metastatic tumors.[4] He examined microscopically not only the tumor tissue but also the blood vessels of non-tumor site. During microscopic examination he found cells in blood vessels had similar morphologic features like cells in the solid tumor tissue.[4]

Almost a century after discovery of CTCs, in 1959, first method for detecting CTCs was discovered via filtration.[5] Later on, in 1960 another method discovered was sedimentation for detecting CTCs.[6] In 1998, immunomagnetic separation technique was discovered for detecting CTCs. Racila et al. also observed that CTCs may be traced early in disease and related directly with disease progression.[7] By using immunomagnetic separation technique, researchers began to detect CTCs by identifying tumor-associated antigens such as epithelial cell adhesion molecules (EpCAM) before and after the start of acancer-specific therapy. Persistence of CTCs after treatment of cancers may be assumed as poor result of the therapy in most epithelial cancers.[5] On the basis of results from large prospective multicentre trials, Food and Drug Administration (FDA) has approved CellSearch® (by Janssen Diagnostics) which became most effective and gold standard technique for extraction and enumeration of CTCs.

Techniques for the enrichment and detection of CTCs

CTC enumeration term used for counting the number of CTCs present in peripheral blood circulation, whereas CTC enrichment is used to separate the CTCs from the non-tumor cells [predominantly white blood cells] in blood sample of carcinoma patients. Previously a lot of researches were done on CTCs enumeration, but now most recent researches are focused on the enrichment and detection of CTCs. Because, only few CTCs are present in blood circulation in comparison to large number of blood cells, so detecting CTCs from the peripheral blood and differentiating CTCs from non-tumor cells and leukocytes, remains technically challenging.

Approaches for CTC Enrichment

CTCs are extremely rare cells in the bloodstream in comparison to normal blood cells, so we can only separate these CTCs from blood of cancer patients with the help of enrichment techniques. There are two types of enrichment method-positive and negative. In positive enrichment, CTCs are directly removed from peripheral blood sample. In Positive enrichment, generally antibodies against the epithelial cell adhesion molecule (EpCAM) are used which is based on antigen- antibody interaction principle. But, Negative enrichment is an indirect process in which non-tumor cells such as leukocytes are targeted and they are separated from the CTCs, to accomplish a CTC-enriched blood sample. CTC enrichment based on the physical properties of CTCs (size, density, electric charges, deformability) and biological properties of CTCs (cell surface protein expression, viability).

Based on physical properties

These are the methods which are based on physical properties: i) density gradient centrifugation; ii) filtration of CTCs which is based on difference in size, through special type of filters such as the ISET (isolation by size of epithelial tumor cells) filter and a 3-dimensional microfilter; iii) based of deformability – CTCs have less capacity to deform in comparison to erythrocytes and leukocytes; iv) a microfluidics device which use both multiorifice flow fractionation and the dielectrophoresis (DEP) cell separation technique.[8,9] Recently, a new device dielectrophoretic field-flow fractionation (DEP-FFF) device is developed which can differentiate between the viable and dead CTC. The viable CTCs show different response to DEP device because, viable cells have different size and membrane properties in comparison to dead CTCs.

Based on biological properties

Immunobead assays

This is based on immunological response using antigen-antibody interactions. There are two types of antibodies used for the separation of CTCs cells and non-tumor cells, one against the tumour cells and another against the common leukocyte antigen CD45. In positive selection antibodies against the epithelial cell adhesion molecule

(EpCAM) or CK 9, CK19 positive cells is used while in negative selection antibodies against CD45. In Immunomagnetic assays an antibody coupled with a magnetic bead is used. The magnetic bead tagged with antibody interact with antigen and forming antigen-antibody complex which can be isolated by applying a magnetic field. Among the all currently available EpCAM-based technologies, the US Food and Drug Administration has approved only Cell- Search® system (Veridex).[8]

Microdevices

At present, researches are focussed on the development of microfluidics devices ("chips"). The first CTC chip which was composed of an array of anti-EpCAM antibody-coated microposts. Now, by using micro-Hall detector which is based on microfluidics chip, even a single CTCs (tagged with a panel of magnetic nanoparticles) in a whole-blood sample can be measured.[10] By using this techniques a huge number of viable CTCs can be isolated in a single sitting from whole blood without using an initial enrichment process.

Nano detectors

These Nano detectors consist of EpCAM antibodies, which can be put into a peripheral vein. Due to this, it comes in directly contact with more volume of blood, thus allowing the capture of greater numbers of CTCs.[11]

Recently, the CTC-iChip is developed which is based on both physical (size) and biologic (immunomagnetic) properties of CTCs. This device is able to categorize rare CTCs in both epithelial and non- epithelial tumors from whole blood at a rate of 10 million cells per second.[12]

Approaches for CTCs Detection

Even after enrichment, the obtained CTCs still have considerable number of non-tumor cells. So, to distinguish the CTCs from leukocytes and other non-tumor cells the following techniques are used.

Flow cytometry-based detection of CTCs

This is most commonly used assays to detect CTCs. In this immunofluorescence-based technique CTCs are positive for both epithelial markers [cytokeratin (CK) and EpCAM] and the nuclear dye 40, 6-diamidino-2-phenylindole (DAPI), but negative for the leukocyte markers (CD45). Major limitation of this technique, unable to differentiate between viable and dead cells. To remove this limitation, EPISPOT assay (Epithelial Immuno SPOT), can be used for the detection of secreted proteins which is secreted only by viable CTCs. So, EPISPOT assay helps in separation of viable cells from apoptotic ones.[8]

mRNA-based detection of CTCs

In this technique, CTCs can be detected by targeting specific mRNAs with reverse transcription PCR (RT-PCR).[13] Another recent advances used for CTC detection is duplex real-time RT-PCR assay. Main advantage of this technique, it had higher sensitivity rate in detection of CTCs in comparison to immunostaining technique, especially in early breast cancer.

Clinical applications of CTCs

The US Food and Drug Administration (FDA) approved Cell Search system for CTC counting in only breast, prostate, and colorectal cancer. Many researchers used this technique (Cell Search) for patient monitoring and to see therapeutic response of various tumours.

Breast Carcinoma

Among the all tumor which are approved by FDA for CTC counting, breast cancer is most favourite to the researchers and most studied. CTC is a well-established prognostic indicator in both metastatic and localised breast carcinoma.[14] CTC is an important biomarker for real-time monitoring of the status of tumor metastasis before and after curative treatment. With the help of CTCs, the status of various receptors like estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2) in breast cancer can be evaluated.[15] Among all, HER2-positive CTCs may be associated with poor prognosis.[16] In some studies, researchers found that HER2-positive metastatic breast cancer cells show EpCAM negative CTCs. Recently in 2018, the German SUCCESS trial was done to observe the beneficial effect on CTCs and overall survival after radiotherapy in localised breast cancer patients.[17]

Prostate Carcinoma

Prostate cancer is the second most common cancer effecting male populations. CTC enumeration has been used as a prognostic implement for metastasis status and overall survival. In patients with early stage prostate carcinoma before radical

prostatectomy, Cell Search shows CTC svery rarely. In the phase II clinical trial CABARESC, the association between androgen receptor splice variant 7(AR-V7) expression in CTCs and resistance to cabazitaxel was observed and it was found there is no association between cabazitaxel efficacy and AR-V7 positive CTCs.[18] The presence of AR-V7 in CTCs was associated with resistance to abiraterone and enzalutamide and correlated with overall bad prognosis.[19]

Colorectal Carcinoma

CTCs have been associated with worst prognosis and increased risk of liver metastasis.[20] CTCs present in colorectal cancer patients with liver or distant metastasis had overall worse prognosis.[21]

Lung Carcinoma

In lung cancer, CTCs detection is very useful because dissemination of tumor cells occurs at early stage and taking biopsies for diagnostic purposes from lung is very tough job. The presence of CTCs in both small cell lung carcinoma and non–small cell lung carcinoma (NSCLC) is indicator of overall worst prognosis.[22,23] The presence of CTCs in NSCLC, indicates involvement of lymph node through metastasis.[24] Higher CTCs counts in lung cancer may be found with larger size tumor and bone metastasis. In both stage I NSCLC and small-cell lung cancer, chemotherapy has been found to decrease CTC number during chemotherapy treatment.[25,26]

Head and Neck Squamous Cell Carcinoma

CTC-positive patients with head and neck squamous cell carcinoma had poor prognosis and it is independent of tumor stage and nodal status.[27]

Thyroid Carcinoma

Thyroid carcinoma CTCs are related with low EpCAM expression.[28] There were no systematic reviews or meta-analyses of CTCs in thyroid carcinoma patients found.

Urothelial Carcinoma

CTCs may be useful to measure the capability of clinical Staging of urothelial cancers.[29] However, overall survival and prognosis related with CTs were less studied.[29] In bladder cancer, the presence of CTCs has also been associated with worse prognosis, while elevated CTC numbers have been associated with recurrence, progression and poor survival.

Melanoma

The main cause of death in patients with melanoma is systemic metastasis of primary tumor, to various organs such as the liver, bone and brain. Since melanoma is not an epithelial tumor, they do not express common CTC markers such as EpCAM or epithelial cytokeratin. Therefore, to isolate CTCs from the blood of patients is really very tough job. Melanoma does not typically express EpCAM; so antigen used for melanoma CTCs are different from carcinoma. The detection of CTCs in melanoma can be correlated with disease stage as well as prognosis. In One study, researcher used chondroitin proteoglycan for immunomagnetic assay of CTCs.[30]

Future aspects

A common shortcoming of the EpCAM-based CTC enrichment systems (CellSearch® system) is, that they are unable to identify epithelial-mesenchymal transition (EMT) which is common process during metastasis. EpCAM-based enrichment methods are unable to identify these type of tumor cells with mesenchymal properties and lacking EpCAM.[31] Future researches should be focussed on developing techniques which can easily identify mesenchymal origin CTCs of various metastatic tumors. So that, CTC assays that can also detect EpCAM-negative tumor cells that might increase diagnostic accuracy in various metastatic tumors.

Future studies can be done to develop different biomarkers for detection of CTCs and tumor DNA. Much more researchs regarding CTCs in many others cancers (e.g. melanoma, pancreatic cancer, head and neck cancer, biliary cancer) are required.

In the future, another application of CTC technology may develop in the field of minimal residual disease diagnostic approaches after completion of curative local treatment. Significant steps ahead are urgently needed to achieve standardised protocols for real-time CTC monitoring and molecular interrogation. Several questions remain unanswered in the field of CTCs diagnostic and metastasis, that which factors are responsible for the generation of CTCs (single or clustered) from a primary tumour or metastatic deposit and what should be implemented to prevent or suppress the haematogenous spread of cancer cells in patients. In future all these unanswered

questions may be resolved for better management of metastatic tumors.

Conclusion

In spite of the large number of researches related with CTCs in various tumor, the full prospective of CTCs in the clinic has not yet been exposed. Recent studies demonstrate the potential of CTCs in screening, early diagnosis, patient stratification, therapeutic approach, predicting relapse, prognosis, therapy resistance, and pharmacodynamics. So, Evaluation of CTCs in the peripheral blood of cancer patients holds great promise in clinical applications. A number of boosting CTC-detection techniques, developed in recent years for CTC isolation and characterisation which are currently not approved as valid diagnostic tools. These new approaches must be validated in multicentre clinical trials with defined goals. We need to develop better approaches which should have potential to isolate and identify subpopulations of tumor cells with down regulated expression of epithelial markers and upregulated expression of mesenchymal markers in various tumours.

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