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Current Status and Prospects of Circulating Tumor Cells in Gastric Cancer

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Abstract
Cancers are a big burden economically, both to families and health care agencies. With trends of increasing cancer incidence, that burden is expected to increase in the foreseeable future. Strides have been made in precision surgery and groundbreaking advances in cancer pharmacotherapy have made living longer with cancer a graspable reality. Thus, diagnosing cancers early with accuracy and lower costs are not just demanded patients' wellbeing, but imperative to reduce the pressure on health care funding. Circulating Tumor Cells (CTCs) are the dissemination of tumor cells through the blood which are originated from the primary tumor lesions. The involution of CTCs differs according to cancer types. The detection of CTCs in peripheral blood of patients with gastric cancers has been detected and it may serve as a blood-based marker for early diagnosis, tumor progression, metastasis as well as recurrence. Several techniques have been developed for the detection of these cells and studies have shown contrasting results using different techniques. This review focuses on the technical advances in different methods of enrichment and detection of CTCs with their clinical significance pertaining to gastric cancers.
Keywords Biomarkers, circulating tumor cells, gastric cancer, tumor prognosis.

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Introduction

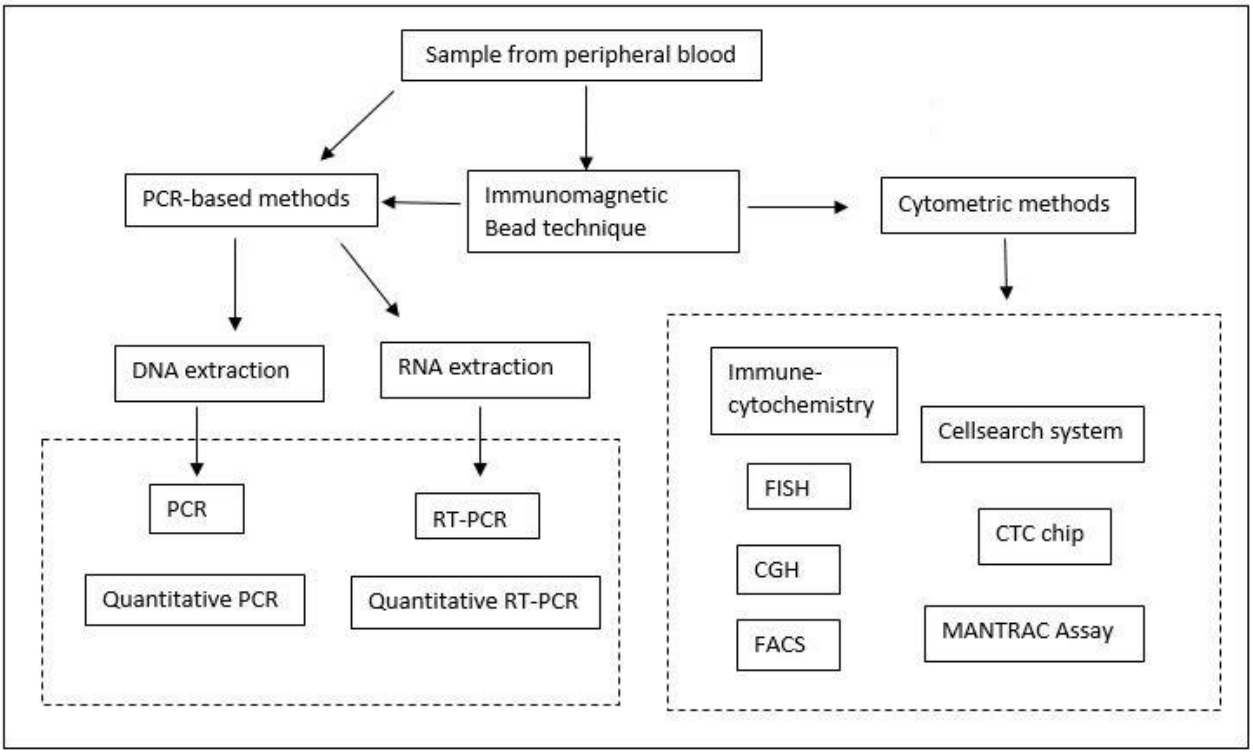
Cancers are challenging and a leading cause of mortality in modern times. Its incidence is expected to grow with the increase in population and changes in lifestyle that potentially increases cancer risk. According to the World Health Organization (WHO), gastric cancer is among the most commonly diagnosed cancers. It is one of the most evil tumors with high illness and death rates (1 million new cases and about 800,000 deaths per year) and 5-year survival rate of less than 30% [1]. Although the diagnosis and treatment of cancers have evolved over the years, but the clinical outcome is still not satisfactory due to diagnosis at late stages. If gastric cancer is found at an early stage, the 5-year survival is roughly 90% [2]. The hurdle is the lack of adequate diagnostic methods for early detection, prognostication and unsatisfactory treatment at advanced stages of gastric cancer. The issue that also needs to be addressed is the high prevalence of metastasis and risk of recurrence. Therefore, to overcome these difficulties, development of new non-invasive, cost-effective diagnostic and monitoring tools is imperative.

Studies regarding the need for minimally invasive or non-invasive methods for identifying gastric cancer have been carried out to discover the most convenient tool to diagnose gastric cancer. Over the past few decades, there have been advances in the field of serum proteomics with much progress made in blood marker detection technology. Circulating tumor cells (CTSs) were first found in 1869 in the peripheral blood of cancer patients, they were defined as tumor cells arising from primary or metastatic tumors circulating in peripheral blood [3]. Studies in gastric cancer patients suggest that CTC-positive events with increased number of CTCs were associated with poor prognosis than CTC-negative patients [4]. Reports as well indicate that measurement of CTCs in gastrointestinal cancer patients could be a useful tool for staging tumor, metastasis, survival, and monitoring the response to therapy [5, 6]. In the present review, we have discussed the recent advances in CTCs in gastric cancer.

Methods for detection of CTCs

In the past few decades, numerous methods have been produced for the isolation / enrichment and detection of CTCs. The molecular characterization of CTCs offers a singular power to access genotypic and phenotypic characteristics of cancer without the

Fig. 1 Representative detection methods of circulating tumor cells [7]. FISH = fluorescence in situ hybridization; CGH = comparative genomic hybridization; FACS = fluorescence-activated flow, PCR = polymerase chain reaction; RT-PCR = reverse transcription polymerase chain reaction; CTC chip = circulating tumor cell chip.



demand for a biopsy. Circulating Tumor Cells appear in very low concentration in peripheral blood of cancer patients and especially CTCs are lower in gastric cancer as compared to other malignancies. Therefore, detection of CTCs with adequate sensitivity and specificity has been a major contest. All the methods consist of isolation and detection phases. As CTCs are very rare in the peripheral circulation (1 CTC/ 1×10^6-1×10^7 mononuclear cells), the isolation or enrichment process is required. The Fig. 1 shows a summary of different detection techniques evolved over time.

Isolation techniques

Isolation of CTCs includes morphological based isolation and immunological techniques. In the morphological based technique, the size, density and electric charges are taken into consideration; whereas, the immune-magnetic technique is a magnetic bead-based separation method.

Cell morphology-based isolation

In this process, the isolation of CTCs is sized based and it does not require functional modification or complex enrichment procedures. It is also known as ISET (isolation by size of epithelial tumor cells), which works as a micro filter to isolate CTCs. Its isolation sensitivity is approximated to one tumor cells per millimeter [8]. The most valuable advantage of this technique is its capability to isolate CTCs without destructive cell morphology and further enabling immune-cytochemical or immunofluorescence evaluation. The alternative technique to separate CTCs is density gradient separation which uses Ficoll-Hypaque. The ficoll-hypaque procedure can be contaminated, so later OnkoQuick was developed to avoid the cross contamination of different layers by employing a porous membrane resulting in the higher recovery rate of CTCs [9]. Recently, a negative selection based method called RosetteSep™ *(*Stem cell Technologies) was developed to improve the specificity of standard gradient separation [10, 11].

Immunomagnetic enrichment

This is one of the most used methods these days, it works by targeting specific bio-markers that are expressed on CTCs. Currently, it can be done using

two different strategies. One is using the epithelial cell-specific marker, e.g., EpCAM (epithelial cell adhesion molecule) and CK (cytokeratins) expressed by tumor cells from the epithelial origin. Another is using tumor-specific markers (α-fetoprotein, CEA (carcinoembryonic antigen), Her2-neu, MUC1/MUC2, and mammaglobulin) expressed by a specific type of cancer cells [12]. Immuno-magnetic isolation utilizes monoclonal antibodies labeled magnetic microbeads and by magnetic force separates CTCs from the background of leucocytes, an anti-CD45 negative selection of leucocytes such as MACS™ (magnetic activated cell storing system) enforces the sensitivity of isolation. The positive aspect of this technique is that it prevents cell lysis during CTCs being isolated, which allows the following CTCs count, immunochemistry and immunofluorescence assays.

According to a study which compared four CTC detection technique (MACS™, RosetteSep™, OnkoQuick™ and OnkoQuick ™plus), CTC recovery rate was higher with high reproducibility and accuracy with the MACS system than the rest [13]. CellSearch Systen™, a leading semi-automated immunomagnetic separation system, is the only Food and Drug Administration (FDA) approved system for the detection and analysis of CTC routinely in patients with colorectal cancer, metastatic breast cancer and prostate cancer [14]. The drawback of this method is that this method can only detect selected CTCs. There are many new advanced technologies (e.g., CTC-Chip, CAM and NV1066), but each has their own strengths and limitations and are constantly evolving.

Identification techniques of CTCs

After the isolation of CTCs, further investigation about the origin and profiling is carried out. Currently, there are two major techniques for the identification or enrichment of CTCs: nucleic acid method and cytometric method.

Nucleic acid based methods

So far RT-PCR (Reverse Transcriptase Polymerase Chain Reaction) based technique is considered to be more sensitive. A more advanced RT-PCR approaches have been in use to screen more than one single biomarker simultaneously. The advantage of RT-PCR is that some trained personnel can manually interpret, identify CTCs in mRNA level and also facilitate target genes search in relation to metastasis. However, there are few drawbacks such as contamination that could occur during sample preparation with non-malignant cells, amplification of cell-free nucleic acids and false positive results can occur. Therefore, it is valuable to select the appropriate technology considering the type of cancer being studied and desired downstream analysis.

Cytometric based methods

Cytometric based techniques identify and count the CTCs using monoclonal antibodies targeting specific markers. As compared to RT-PCR technique, it not only gives information relating to subpopulation quantification, but also highly specific and the data obtained is statically more precise. Fiber optic array scanning technology (FAST) is a fast and precise CTC locating system equipped with a large field of view [15]. It detects CTCs without an enrichment procedure and minimizes cell loss. In addition, several scanning systems are available in the market such as EPISPOT (epithelial immunospot), ACIS (automated cellular imaging system) and ARIOL (Applied Imaging corp., Germany).

Clinical role of CTCs in gastric cancer

Since the detection techniques of CTCs are on the rise, there have been a number of studies that have shown the clinical relevance of CTCs in gastric cancer. In practice, the detection of CTCs is thought to be valuable in early detection of gastric cancer, monitoring of treatment responses, disease recurrence, and progression. There are several methods of detection of CTCs, yet identifying the best method and marker for detection of CTCs still remains unclear. The detection rate of CTCs changes according to the methods used, i.e., RT-PCR or non-RT-PCR methods. Individual studies have shown that for same markers, there were different detection rates using different methods [16].

In an analysis of Tang et al. [17], it has been shown that CTC detection independently cannot be employed as a masking tool for gastric cancer; nevertheless, it can be applied as a noninvasive method for confirming a gastric cancer diagnosis. According to another study, the sensitivity of CTCs was found to be inconsistent and low for detecting gastric cancer [18]. In another study of Kolostova et al. [19], it was reported that CTC-positive rates correlated with the disease stage as well as lymph

Table 1 Correlation of circulating tumor cells (CTCs) detection and survival in gastric cancer.

Method	CTC detection rate	Clinical importance	Study
Multiplex RT-PCR	68%	Correlation with recurrence	Wu et al. [24]
RT-PCR	40%	Overall survival	Illert et al. [25]
RT-PCR	15%	Overall survival	Koga et al. [26]
RT-PCR ELISA	44%	Disease free survival	Yie et al. [27]
RT-PCR	45%	Disease free survival	Saad et al. [28]
Cell Search®	91%	Predictive marker for progression-free survival	Matsusaka et al. [29]
RT-PCR	46%	Disease free survival	Cao et al. [30]
real-time PCR assay	55%	predictive marker	Bayat et al. [31]
MetaCell®	59%	Prognosis & therapeutic response	Kolostova et al. [32]

node participation. There have been several studies regarding CTCs as a biomarker for disease progression and micro-metastasis. RT-PCR has been the most widely used method and is regarded to be the most sensitive method reported. Nevertheless, it is time-consuming and effortful to apply in clinical diagnosis [20, 21]. It is likewise reported that the postoperative incidence of recurrence in advanced tumor stages is higher as compared to early stages and the hematogenous dissemination is the most likely kind of tumor recurrence [22]. Hence, CTCs are important contributors and indicators for metastasis and relapse of the tumor.

Table 1 presents some studies on CTCs detection by various techniques and their significance in gastric cancer patients. A clear conclusion cannot be drawn from these studies; larger studies will be needed to reach a conclusion. At present, the detection of CTCs alone cannot be suggested as a monitoring tool for gastric cancer. In a study of 251 patients with diagnosed gastric adenocarcinoma, CTC detection was done using cell search system. It showed that cell search system can detect CTCs sensitively. The general persistence rate was significantly lower in patients with CTCs than in those without CTCs. The detection of CTCs may be a useful tool for predicting tumor progression, prognosis, and the effect of chemotherapy in patients with gastric cancer. It also showed that the patients in whom CTCs were detected had a significantly higher relapse rate compared to patients in whom CTCs were not detected [23].

Conclusions

At present, cancer claims to be the major cause of morbidity and mortality, it is required to counter it by developing and devising a fast and easy diagnostic, predictive and monitoring method. Since the first detection of CTCs in the blood of cancer patients, it has been a very important tool for different cancers. In case of gastric cancer, the role of CTCs has been less as compared to others. One of the reasons may be that majority of gastric cancers is developed from the mucosa, which has less vascularization as compared to other solid tumors. The presence of CTCs in gastric cancer in serum is very low. There have been several studies that have shown CTCs can be isolated from the gastric lavage. The focus at present is more on the detection and isolation of CTCs. There needs to be more studies before CTC technologies can be used routinely in practice.

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Conflicts of interest

There are no conflicts of interest.

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