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Possible applications of circulating tumor cells in patients with non small cell lung cancer

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ABSTRACT

Recent experiences indicate that, as already reported for other types of cancer, circulating tumor cells (CTCs) may play a role also in non small cell lung cancer (NSCLC) for diagnosis, therapy monitoring and prognostic purposes. CTCs evaluation could be particularly relevant in this clinical setting not only for the objective difficulty in obtaining tumor tissue, but also because of the lack of reliable tumor markers. In the current review, we will focus on the possible applications of CTCs in NSCLC patients.

1. Introduction

Circulating tumor cells (CTCs) are epithelial cells that can be found in the bloodstream of patients with various malignancies and that are not detected in healthy subjects [1] (Fig. 1). CTCs are extremely rare occurring at a frequency of 1 CTC per 10^6–10^7 leukocytes and may also form aggregates, known as circulating tumor microemboli (CTM). Over the last few years several methods have been developed with the aim of detecting CTCs, though the CellSearch® (CS) represents the only diagnostic assay approved for this purpose by the American Food and Drug Administration (US FDA). It has been demonstrated that CTCs isolated from small cell lung cancer patients can form tumors in immunocompromised mice with preserved morphological and genetic characteristics, providing evidence for their tumorigenity [2]. Recently, Morrow et al. demonstrated tumorigenicity of CTCs to generate a patient CTC derived explant (CDX) [3]. Moreover, Chinese researchers reported that CTCs from an individual cancer patient exhibit reproducible copy number variation (CNVs) patterns similar to those of the metastatic tumor of the same patient [4]. Therefore, the authors hypothesized that CNVs at certain genomic loci are the key events of metastasis and that CTCs might be involved in the metastatic process.

In cancer patients the detection, enumeration and molecular characterization of CTCs could represent an useful tool to establish the diagnosis, the prognosis or to perform an early assessment of disease progression. The prognostic significance of CTCs has already been proved in metastatic breast, colorectal and prostate cancer [5–7].

Furthermore, recent experiences indicate that CTCs could play a role also in non small cell lung cancer (NSCLC) for diagnosis, biological characterization and disease monitoring [8,9]. CTCs could be particularly useful in this clinical setting since tumor tissue obtained from biopsies is often insufficient for a comprehensive histopatological-molecular analysis, patients are not always suitable to receive multiple biopsies and because of the lack of reliable tumor markers.

In the current review we will focus on the main detection methods, molecular characterization and prognostic significance of CTCs in patients with NSCLC.

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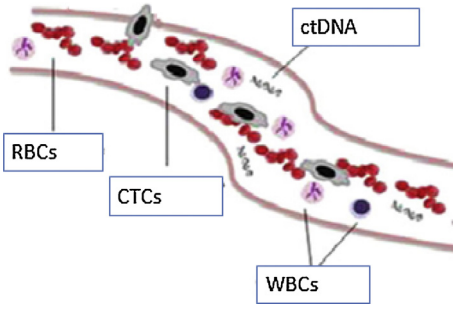

Fig. 1. CTCs are cancer cells that detach from the tumor into the peripheral blood (modified from Rolfo et al. [11]).
CTCs: circulating tumor cells; ctDNA: circulating tumor DNA; RBCs: red blood cells; WBCs: white blood cells.

2. Detection methods of CTCs

Several approaches have been developed to detect CTCs ranging from nucleic-acid-based methods to size based systems or methodology based on the application of immune-magnetic beads [10,11].

Initially, nucleic-acid-based methods using reverse transcriptase polymerase chain reaction (RT-PCR) were widely used to infer the presence of CTCs based on the detection of specific DNA or RNA sequences differentially expressed by tumor cells [10]. However with these methods, based on the assumption that CTCs represent the source of mRNA transcripts, CTCs cannot be visualized for morphology or enumeration. For these reasons there has been a subsequent shift towards cytometric methods.

CS (Janssen Diagnostics, Raritan, NJ, USA) and the isolation by size of epithelial tumor cells (ISET, RareCell Diagnostics, Paris, France), a biological and a physical method, represented up to now the most commonly used techniques for CTCs detection in NSCLC (Figs. 2 and 3). The CS system uses an unique technology to capture, isolate and enumerate CTCs from 7 to 5 mL blood sample. This CTC detection system is based on a combination of immunomagnetic labeling and automated digital microscopy. In particular, epithelial cells are enriched from whole blood by magnetic nanoparticles coated with an anti Epithelial Cell Adhesion Molecule (EpCAM) antibody; the enriched sample is then labeled with fluorescent anticytokeratin antibodies, with the leukocyte marker CD 45 and with the fluorescent stain DAPI (4′,6-diamidino-2-phenylindole). Subsequently, a semi-automated fluorescence-based microscopy system permits a computer-generated reconstruction of cellular images. The CTCs identification is based on the following criteria: cytokeratins positivity, nuclear staining by DAPI and lack of CD45 expression [12,13]. The CS system can detect one CTC per 7.5 mL of peripheral blood with high reproducibility, although there could be false positives due to labeling of the non-tumor cells or false negatives from CTCs depleted of specific antigens [14]. In particular, detection of CTCs through the CS system is dependent on tumor cell expression of EpCAM, an epithelial cell marker; thus, CTCs may be missed by CS since they are less likely to express high levels of EpCAM in the process of epithelial to mesenchimal transition (EMT), which results in down regulation of epithelial proteins such as EpCAM. However, the role of EMT in metastatic process *in vivo* is still matter of debate, as it was recently reported that EpCAM-negative epithelial cells are not associated with poor outcome in NSCLC [15]. Recently, British researchers published a clinical case study of a patient with advanced EGFR wild type and ALK non-rearranged NSCLC [3]. They demonstrated, for the first time, tumorigenicity of CTCs to generate a patient CDX. It's interesting to note that examination of blood samples revealed absence of CTCs using the CS, whereas size based CTC enrichment revealed abundant CTCs, most of which were positive for mesenchimal markers. The authors concluded that the absence of NSCLC CTCs detected by CS does not preclude CDX generation highlighting the role of the EMT process and mesenchymal CTCs in the metastatic process.

Methods to capture EpCAM-negative CTCs from blood samples, based on the employment of immunomagnetic beads coated with specific cancer related markers such as CD146, are being investigated [16]. In any case, the CS system is the only technology approved by the US FDA for the enumeration of CTCs in the whole blood of metastatic breast, colon and prostate cancer patients.

Another technology, named CTC-chip system, uses a microfluidic platform consisting of an array of 78,000 microposts coated with anti-epithelial cell adhesion molecule (anti-EpCAM) antibodies to capture CTCs under laminar flow conditions [17]. Also for this method exists the limitation of being useful only for EpCAM positive cells. However, CTC-chip seems to have a higher CTC detection rate respect to CS, probably due to a lower loss of CTCs during the CTC-chip procedure [18].

Chinese researchers developed the so-called NanoVelcro assays, in which capture agent (e.g. anti-EpCAM)-coated nanostructured substrates were employed to detect CTCs with high efficiency [19]. A recent experience conducted in a series of NSCLC patients showed that NanoVelcro platform is able to capture more CTCs than CS and that in CTCs enriched by this system ALK-status can be evaluated effectively [20].

The ISET method works as a microfilter that isolates CTCs based on their size discrepancies, without a previous immune-based selection. As tumor cells are larger (>8 μm) than leukocytes, blood samples are filtered through membranes with calibrated pores 8 μm in diameter. Enriched cells are stained on the filter for cytomorphological or further analyses. This system can capture CTCs as individual cells and as cell clusters or CTM with a claimed sensitivity of one CTC per mL of whole blood [21]. Unlike CS, the ISET method permits to detect both CTCs with absent EpCAM expression and CTM; however, using the ISET method small CTCs may escape detection, while larger leukocytes may be retained. Main differences between CS and ISET platforms are summarized in Table 1.

Few data exist in literature about a direct comparison between CS and ISET in NSCLC. Hofman et al. evaluated CTCs using both the CS and the ISET method in 210 NSCLC patients undergoing radical surgery [22]. In this study, CTCs were detected in 69% of patients using CS and/or ISET and in 50% and 39% of patients using ISET and CS, respectively; therefore, the authors concluded that CS and ISET should be considered as complementary methods for CTCs detection. Another comparison study of CS and ISET was carried out by Farace who enrolled 60 patients with metastatic breast, prostate and lung cancer [23]. The authors reported significant discrepancies, mostly dependent on the type of cancer, between the numbers of CTCs enumerated by both techniques and concluded that CS may present important limitations, especially in patients with metastatic NSCLC probably due to the number of tumor cells involved in the EMT process. Krebs et al. compared both methods in 40 chemo-naïve stages IIIA-IV NSCLC patients [24]. In this study the ISET system was able to detect CTCs in 80% of the patients compared with the 23% identified by CS; moreover, circulating tumor microemboli were observed in 43% of the patients using ISET, while were undetectable by CS. Differences in CTC detection reported with CS and ISET could be related to the evidence that the CS system is able to identify only EpCAM-positive cells and then might be inadequate to identify CTCs that undergo EMT. However, as suggested by some authors, both methods could play a complementary role in CTCs detection [22].

More recently, a new strategy of capturing CTCs for downstream molecular analysis renewed the landscape of methods suitable to investigate CTCs. The GILUPI CellCollector (DiagnostikNet, Berlin, German), as a catheter based or wire-based enrichment of EpCAM-positive cells that could be use in vivo, recently it was used to