CIRCULATING TUMOR CELLS: FASCINATING INSIGHTS IN THE CLINICAL RELEVANCE

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ABSTRACT

During tumor progression, circulating tumor cells (CTCs) shed from epithelial tumor and circulate through bloodstream to form metastasis at distant sites. CTCs are heterogeneous rare cells that hold the promise of a valuable prognosis and prediction biomarker. The detection and analysis of CTCs through enumeration provide significant information on monitoring in real time the therapy efficiency and selection of personalized therapy. A continuous improvement of the detection techniques is still required. Therefore, this review focuses on significance of CTCs clinical approaches in the personalized treatment.

Keywords: circulating tumor cells, enumeration, personalized treatment

REZUMAT

In timpul progresiei tumorale, celulele tumorale circulante (CTC) se desprind din tumora epitelială și circulă în fluxul sangvin pentru a forma metastaze la distanță. CTC-urile sunt celule rare heterogene, care dețin rolul promițător de biomarkeri prognostici și predictivi. Detecția și analiza CTC-urilor prin numărare oferă informații importante în timp real despre eficiența terapiei și ghidarea terapeutică. O continuă îmbunătățire a tehnicilor de detecție este încă necesară. Prin urmare, acest articol se focalizează pe importanța CTC-urilor în abordările clinice din cadrul tratamentului personalizat.

Cuvinte-cheie: celule tumorale circulante**,** numărare, tratament personalizat

INTRODUCTION

Circulating tumor cells (CTCs) in the peripheral blood were observed by Ashworth, T.R (1869) for the first time in a metastatic patient as particular cells that look similar to tumors. In addition, *Paget* claimed that tumor cells "seeds" can form secondary metastasis on predisposed certain "soil", predominantly liver and bone [1]. However, the nature of CTCs and their role in the mechanism of metastasis process were unknown.

Over one hundred and forty years of cancer research, the involvement of CTCs in the metastatic cascade [2] as well the characteristics of CTCs that illustrate partially the tumor heterogeneity were revealed [3]. Overall, CTCs exhibit strong interest not only in the knowledge of mechanisms underlying metastasis process, but also in the development of non-invasive clinical approaches for monitoring tumor evolution.

The conventional tissue biopsy is a robust approach used in diagnosis of cancer stage and disease assessment, but it is unfeasible to provide information on spatial and temporal pattern of tumor progression. In addition, the imaging provides limiting information on tumor while radiological techniques show high health risks. Moreover, serological tumor markers frequently used in clinical monitoring of tumor progression during treatment exhibit lack of sensitivity and specificity [4]. An alternative to conventional clinical approaches is liquid biopsy through analysis of CTCs in blood sam-

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ples providing direct real-time insights on tumor evolution with minimal invasiveness, that could be repeated any time, with very limited interventions and without exposing the patient to health risks [5]. Therefore, CTCs hold the promise of a valuable biomarker in prognosis and prediction of treatment efficiency, early relapse and therapy selection.

CTCs were exploited in the clinical practice following the development techniques with appropriate performance characteristics (e.g. sensibility, specificity, reproducibility). CellSearch is the only FDA approved detection system used for clinical detection of CTCs validated for metastatic breast, colon and prostate cancer [7]. However, low concentration and heterogeneity hamper the detection of CTCs [6]. A continuing improvement of techniques performances is required.

In the present review, we describe the characteristics and detection techniques relevant to clinical approaches of CTCs, with a focus on the clinical utilities of CTCs as prognostic and predictive biomarkers in personalized medicine.

Physical and molecular properties of CTCs

CTCs occur at low concentration of 1CTC per 10^6 - 10^8 blood cells [8, 9]. Detection of CTCs for various metastatic cancer types, including ovarian, lung, pancreatic revealed wide ranges of CTCs frequencies from 0 to thousands of cells [10]. In contrast, the presence of CTCs in healthy or nonmalignant patients does not exceed 1 CTC in a very limited number of patients. On the other hand, the 0 CTCs in metastatic carcinoma patients could be addressed to infrequency or limitation of separation/detection techniques. Therefore, improvement of techniques sensitivity is further required.

In addition, CTCs exhibit a strong dynamic in the morphological and phenotypical innate heterogeneity during the disease progression [11]. CTCs diameters range from 9 to 30 μm as intact single cells, clusters, apoptotic and cells fragments with round or odd shapes [12, 13]. In general, patients exhibit single CTCs, but the simultaneous presence of single CTCs and clusters are detectable in patients with advanced stages. However, the presence of CTCs clusters in circulatory system is associated with malignancy [14, 15]. There are studies suggesting that CTCs use platelets, fibroblats or leukocytes to aggregate in clusters creating protection against immune surveillance [16].

In addition, apoptotic CTCs phenotype ($CK^+/M30^+$) is present in a significant percentage in early or metastatic stage of breast cancer [17]. The changes in apoptotic CTCs levels before and after treatment in patients with small cells lung cancer was related independently to efficiency of treatment [18]. Moreover, numerous immunocytochemical studies on CTCs from various carcinomas confirmed the presence of large CTCs with typical high nucleus to cytoplasmic ratio [19, 20]. Morphological features of tumor cells (nucleus size and shape) are fundamentally correlated with distant metastasis in pathology [21]. Further classification of CTCs based on their nucleus size demonstrated that the number of the smallest size nucleus CTCs was successfully correlated with visceral metastatic prostate cancer [22]. However, the presence of very small size nucleus CTCs should be also addressed to wider number of cancers. In respect to molecular profile, CTCs co-overexpress epithelial molecule of adhesion (EpCAM) and cytoskeletal maker cytokeratine (CK). However, the present markers show a low specificity being expressed also in normal epithelial cells. Nevertheless, EpCAM and CK are routinely used in the clinical pathology in combination with inspection of morphological structure of the cells. During metastasis process, CTCs show a transition in their phenotype. Throughout the epithelial-mesenchymal transition mechanism, the mesenchymal markers (e.g. Vimentin) are upregulated while the epithelial markers are suppressed. Interestingly, experimental studies revealed a partial transition between epithelial and mesenchymal phenotype that came out to three phenotypes: epithelial, epithelial-mesenchymal and totally mesenchymal phenotypes [23]. Surprisingly, these phenotypes of CTCs demonstrate clinical relevance. For example, analysis of phenotypic populations of CTCs (CK^+/Vim^- vs CK^+/Vim^+)

during post 1-cycle pazopanib treatment in small cells lung cancer patients is considered relevant for monitoring the treatment efficacy [24].

Detection of CTCs

The detection of low abundance CTCs requires a-priori enrichment or separation of CTCs from the peripheral blood cells.

The gold standard for detection and enumeration of CTCs, CellSearch, is based on EpCAM immunomagnetic enrichment method. The enriched EpCAM positive cells are confirmed as being epithelial tumor cells whether cells express CK8, 18/19 while lack CD45, the leukocyte common antigen marker. Several studies showed that CellSearch detected successfully the CTCs counts associated with prediction and prognosis of the patient outcome during therapy in metastatic breast, colon and prostate cancer [25, 27]. However, CTCs change their phenotype lacking EpCAM during EMT process. Hence, a specific antibody or label free method should be employed in separation of CTCs. Multiple technologies based on physical and molecular properties of CTCs were developed. For example, Fluxion technique uses cocktail of antibodies against epithelial and mesenchymal phenotypes demonstrating an improved number of CTCs [28]. In addition, methods based on physical properties, especially size based filtration exhibit high sensibility in clinical practice. For instance, separation of CTCs based on ISET technology (ISET: Isolation by SizE of Tumor cells) demonstrated detection of 1 CTC in the peripheral blood [29]. Comparison of CTCs enumeration through ISET vs CellSearch technologies in patients with non-small lung cancer showed a higher number of negative EpCAM CTCs separated by ISET [30]. Therefore, CellSearch system could underestimate the number of CTCs. Currently, microfluidic based platforms with increased sensibility have been presented [31]. Particularly, a new CellMax biomimetic platform (CMx) was developed to investigate CTCs in early stage. The new platform was tested only in a single center that further needs to be validated by independent groups.

CTCs Clinical Relevance

The presence of CTCs in the peripheral blood is associated with poor patient outcome.

In addition, CTCs counts are considered clinical prognostic biomarkers in the stratification of patients in groups that follow treatment response and predict the patient survival outcome before and after treatment [32]. For example, the study of preoperative non-small lung cancer patients with resectable tumors showed a shorter overall survival and poor prognosis for patients that exhibit higher CTCs number over the cut-off point (threshold). The presence of CTCs significantly correlated with poor prognosis is confirmed in other cancers, such as breast cancer [33] and pancreatic cancer [34].

In addition, CTCs enumeration is commonly used in clinical practice to monitor the therapy outcome and to select the appropriate therapy suitable to each patient.

For example, the dynamics of CTCs numbers for metastatic breast, colon and prostate cancer predicts the overall survival and progression free survival related to real time treatment response. The patients with a conversion from CTCs counts ≤5 cut off point to CTCs counts ≥5 above cut off point or patients with persistent CTCs before and after therapy showed a worse survival. During the therapy course, CTCs counts ≤5 indicate treatment efficiency through a significant improvement of patient outcome [35]. Moreover, changes in CTCs counts in response to chemotherapy were monitored in metastatic breast cancer [36]. Therefore, CTCs number is the strongest predictor of patient outcome during treatment, a useful tool to monitor in real time the treatment efficiency.

The populations of patients that show an increase or constant number of CTCs can be resistant to therapy [37]. Moreover, it has been recently shown that CTCs enumeration plays a significant role in therapy selection [38].

Although the prognostic and predictive roles of CTCs are proven in metastatic patients, the detection of CTCs in non-metastatic patients is quite challenging. In the early stages, the number of CTCs is extremely low < 5CTCs

in 10mL blood [39]. Nevertheless, Bidard *et al*. [40] succeeded to detect 1CTC in 7.5mL blood, but it is still unclear whether 1CTC is clinically relevant.

CONCLUSIONS

Circulating tumor cells represent multi-functional biomarkers, valuable in monitoring the progression of tumor in real time related to patient outcome and treatment efficacy in a non-invasive way and in prediction of patient response to therapy.

CTCs based identification and enumeration associated with progression free survival and overall survival profiles of patients offer the possibility to change the therapy in short period of time instead of waiting for long time conventional clinical approaches to check the progression. Moreover, analysis of CTCs can predict a targeted therapy with a best response for each individual patient.

Further studies should focus on basic investigations of CTCs nature, necessary to improve of CTCs detection technologies and thereby, to complement current diagnostic tests.

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