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Circulating tumor cells in breast cancer—current status and perspectives

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ABSTRACT

The phenomenon of tumor cell dissemination through the blood stream has been known since the 19th century. Circulating tumor cells (CTCs) may be detected in peripheral blood of patients with breast cancer and may serve as a surrogate marker for minimal residual disease. Prognostic relevance of CTCs has already been demonstrated in early and metastatic breast cancer and commercially available detection systems are currently employed in various clinical trials. Since peripheral blood is an easily accessible compartment, serial reevaluation of CTCs is possible and may contribute to better therapy monitoring. Another potential of CTCs lies in the characterization of tumor cells. Expression profiles may differ between CTCs and primary tumor, which may result in different responses to treatment. Assessment of molecular features of CTCs may be an important step for the optimization of adjuvant and metastatic systemic therapy.

1. Introduction

The phenomenon of hematogenous dissemination of malignant cells shed from solid tumors has been explored by several researchers in 19th century (Ashworth, 1869; Paget, 1889). Single cancer cells may leave the primary tumor early in the course of disease, disperse through the body via blood stream and serve as precursors of subsequent metastatic growth at secondary organs. The features of the tumor cell, the microenvironment at the 'homing site' and the interaction between those two are crucial for understanding the mechanisms governing the metastatic cascade.

Abbreviations: BC, breast cancer; BCSS, breast cancer-specific survival; CTC, circulating tumor cell DDFS; DFS, disease-free survival; DMFS, distant metastasis-free survival; DTC, disseminated tumor cell; EpCAM, epithelial cell adhesion molecule; HER2, human epidermal growth factor receptor 2; MFS, metastasis-free survival; MRD, minimal residual disease; OS, overall survival; PFS, progression-free survival; RT-PCR, reverse transcription polymerase chain reaction.

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Evaluation and characterization of cancer cells in the bone marrow and blood has become a major focus of translational oncologic research in the last two decades. Circulating tumor cells may be detected in most solid tumor of epithelial origin, but no cancer entity has been studied in this context as extensively as breast cancer.

First data on the prognostic relevance of minimal residual disease (MRD) were provided by the analysis of bone marrow aspirates from breast cancer (BC) patients. In 2005, a large meta-analysis of more than 4700 patients with early BC showed that presence of disseminated tumor cells (DTCs) in bone marrow is associated with poor clinical outcome (Braun et al., 2005). Since one important disadvantage of bone marrow sampling is the invasiveness of the procedure, subsequent studies focused on the easily accessible circulating tumor cells (CTCs) in peripheral blood. The following review will address the current role and future potential of CTCs as a diagnostic tool in early and metastatic breast cancer.

2. Methods for detection of CTCs

CTCs have to be detected among a background of a huge number of blood cells. Therefore challenges in detection and enrichment of CTCs are mainly due to this low frequency and to the heterogeneity of CTCs which is directly correlated to the heterogeneity of the primary tumor. Current technologies refer to these two challenges. Beside the enumeration of CTCs, whose clear prognostic relevance could be shown in multiple clinical studies (Cristofanilli et al., 2005; Rack et al., 2014), characterization of CTCs and therefore the identification of surrogate markers for therapy prediction and monitoring has reached the focus of attention in current research. Characterization on single cell level is the means of choice to increase the amount of information which can be gained from one single liquid biopsy. To resemble the heterogeneity of the primary tumor the amount of analyzed single cells has to be increased. The pathway from sample to information contains the single steps of enrichment, isolation and characterization of CTCs. Besides the "classical" taking of a blood sample there are novel methods to increase the blood volume to be analyzed. Diagnostic leukapheresis uses standard leukapheresis conditions for extracorporeal separation of mononuclear cells, thus increasing the analyzed peripheral blood volume and therefore the number of CTCs. Identification and characterization of CTCs can be done according to standard procedures (Fischer et al., 2013). Another innovative technology to increase the analyzed blood volume is an EpCAM-antibody (Epithelial cell adhesion molecule) activated medical wire (CellCollector™) which remains in the cubital veins of patients for 30 min. EpCAM-positive CTCs could successfully be isolated using this technology (Saucedo-Zeni et al., 2012).

Due to the heterogeneity of CTCs and therefore due to the lack of a universal tumor cell marker enrichment and isolation of CTCs are often combined. There are different approaches to enrich CTCs which can be roughly divided into label-independent technologies using morphological features of the cells like size or density and label-dependent technologies using immunologic characteristics like the expression of certain epithelial or mesenchymal markers (Broersen et al., 2014). Label-independent technologies to enrich CTCs are mainly based on size (filtration, e.g. ISET®, Parsortix, ScreenCell®) (Lin et al., 2010; Freidin et al., 2014; Kulemann et al., 2015), on density of CTCs (Ficoll, e.g., OncoQuick™) (Muller et al., 2005) or on microfluidic characteristics of CTCs (e.g., DFF-chip, JETTA™) (Riahi et al., 2014; Hou et al., 2013). An exceptional position in the different technologies to enrich CTCs holds the CTC iChip, which combines hydrodynamic cell sorting and EpCAM-based selection or negative depletion (Ozkumur et al., 2013). Label-dependent technologies are based on the detection of antigens to distinguish between blood cells and CTCs. All label-dependent technologies benefit from their specificity. On the other hand this specificity includes the disadvantage to enrich and isolate subpopulations of CTCs. For example cells undergoing epithelial-mesenchymal transition (EMT) could possibly be missed. During this process which is considered to be an important step in the metastatic cascade cells change their phenotype, losing epithelial and gaining mesenchymal characteristics (Krawczyk et al., 2014). The majority of label-dependent technologies to enrich CTCs are currently based on the detection of EpCAM. The current "gold standard" and only system approved by the FDA (Food and Drug Administration) is the CellSearch® system. Clinical relevance of the enumeration of CTC could first be shown using this system (Cristofanilli et al., 2004; Bidard et al., 2014a). The CellSearch System combines semi-automated enrichment of EpCAM-positive cells using magnetic nanoparticles and characterization of CTCs which is enabled by immunofluorescent staining of cytokeratin 8, 18 and 19 as well as CD45 to exclude leukocytes and DAPI to stain nuclei. Other label-dependent technologies using the detection of EpCAM are based on immunomagnetic beads (e.g., Adnagen, Isoflux, MACS) (Fehm et al., 2009; Harb et al., 2013) or on microfluidics (e.g., CTC/Herringbone chip) (Nagrath et al., 2007; Stott et al., 2010). Since current detection techniques do not distinguish between viable and apoptotic tumor cells, the ELISPOT assay may be applied to detects proteins secreted from single epithelial cancer cells (Ramirez et al., 2014).

3. Clinical role of CTCs

Clinical value of CTC detection differs between metastatic and early breast cancer. Both the presence of CTCs and their immunocytochemical/molecular features are currently being evaluated in several clinical trials (Table 1).

3.1. Clinical role of CTCs in metastatic breast cancer

Despite an early diagnosis and adequate initial treatment, long-term systemic recurrence rate in breast cancer patients is still estimated at 20–30% (Early Breast Cancer Trialists' Collaborative Group, 2005). Since metastatic breast cancer (MBC) remains incurable, the main aim of therapy at this stage of disease is to maximize the quality of life and improve survival by preventing or slowing tumor progression and to minimize therapy side effects. It is therefore essential to develop treatment regimens that are able to reduce tumor burden by targeting the metastatic site. However, the majority of therapeutic decisions in MBC are still based on the biology of the primary tumor even though the phenotypic and genotypic discrepancy between primary tumor and solid metastases has been reported in several studies (Fehm et al., 2009, 2008; Solomayer et al., 2006; Banys et al., 2012; Krawczyk et al., 2009). CTCs can be detected in peripheral blood of 40-80% MBC patients and represent an independent negative prognostic factor for progression-free survival (PFS) and overall survival (OS) (Table 2). Moreover, changes in CTC levels seem to reflect therapy response in this collective (Budd et al., 2006; Hayes et al., 2006). In this context CTCs can be considered a valuable non-invasive tool to characterize the status and biology of metastatic disease and an interesting alternative to serial biopsies of metastatic lesions.

3.1.1. Prognostic value of CTCs

Prognostic significance of CTCs in MBC was first demonstrated by Cristofanilli et al. in 2004 (Cristofanilli et al., 2004). In this multicenter prospective analysis CTC levels were estimated in peripheral blood (PB) of 177 MBC patients before beginning a new treatment regimen and at the first follow up visit and correlated with survival data. Detection of at least 5 CTCs versus less than 5 CTCs/7.5 ml