ZNACZENIE KLINICZNE KRĄŻĄCYCH KOMÓREK NOWOTWOROWYCH W RAKU PIERSI

CLINICAL UTILITY OF CIRCULATING TUMOR CELLS IN BREAST CANCER CASES

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Streszczenie: Wobec współczesnych trendów do personalizowania terapii onkologicznych, istnieje ogromna potrzeba poszukiwania biomarkerów specyficznych dla konkretnych nowotworów. Czynniki te umożliwiają optymalny wybór terapii celowanych oraz szacunkową ocenę odpowiedzi na leczenie. Wiele badań wskazuje na pogorszenie rokowania u pacjentów, u których stwierdzono obecność krążących komórek nowotworowych we krwi obwodowej oraz poprawę wyników klinicznych w przypadku ich eliminacji w odpowiedzi na zastosowane leczenie. Krążące komórki nowotworowe są izolowanymi komórkami guza, które dzięki nowoczesnym technologiom mogą być identyfikowane we krwi obwodowej chorych z postacią zlokalizowaną, jak i rozsianą nowotworu, np. raka piersi. Prezentowana praca poglądowa przedstawia dostępną wiedzę dotyczącą krążących komórek nowotworowych we wczesnym i rozsianym raku piersi oraz perspektywy ich klinicznego zastosowania w terapii onkologicznej.

Słowa kluczowe: rak piersi, krążące komórki nowotworowe, tranzycja nabłonkowo-mezenchymalna, mechanizmy przerzutowania, kaskada metastatyczna, hipoteza „ziarna i gleby", biomarkery nowotworowe

Summary: Scientific attempts to create personalized cancer medicine require the development of tumor-specific biomarkers. These factors enable to optimize selection of targeted therapies and to assess response to treatment. Several studies in different tumor types have shown worse prognosis of patients in whom circulating tumor cells (CTCs) are detected and improved clinical outcomes following elimination or decrease of CTCs after treatment. Circulating tumor cells are isolated tumor cells disseminated from the site of disease in metastatic and primary cancers, including breast cancer, that can be identified and measured in peripheral blood. Technological advances in their detection, isolation, capture and characterization from phlebotomy samples have rendered it easier to evaluate different CTC biomarkers. In this paper we review CTCs in early and metastatic breast cancer and discuss clinical utility for prognosis assessment and monitoring response to therapy.

Key words: breast cancer, circulating tumor cells (CTC), epithelial-mesenchymal transition (EMT) metastasis, metastatic cascade, models of dissemination, 'seed and soil' hypothesis, tumor-specific biomarkers

INTRODUCTION

Breast cancer is the most popular malignancy not only in Poland, but also in majority of countries of the world. The disease affects approximately 200.000 women in the United States alone. It is recognized as heterogeneous disease comprised of several common different phenotypes [2]. Only approximately 5% of all breast cancer patients are primarily diagnosed with incurable disease as a result of screening, increased awareness and consequent early detection [25]. Unfortunately, despite of optimal treatment pattern, 30-40% of patients diagnosed with curable breast cancer eventually die of recurrent disease [20, 27]. It is said that in general more than 90% of cancer deaths result from the development of hematogenously disseminated metastasis [28]. That is why, improved techniques of detection and treatment of metastatic disease are desired.

The term "metastasis" was coined by Recalmer in 1800's, and even then circulating tumor cells have been postulated to be crucial for the process. The presence of CTCs in patients with cancer was first reported in 1868 [3]. Traditionally the development of disseminated disease has been considered as a sequential rather than concurrent process. The current data are challenging this theory [22, 24]. The initiation of metastasis may be a relatively early moment in tumor biology.

CTC BIOLOGY

The term Circulating Tumor Cells encompasses all types of cells, established as foreign entities in the blood, having some cancerous characters. They are generally defined as nucleated cells lacking CD45, which is considered to be a marker of leukocytes and expressing cytokeratin, being an evidence of epithelial origin of the cells, but they are not homogenous. There are different subpopulations of CTCs, like cancer stem cells (CSC), tumor amplifying cells (i.g. progenitors) and tumor initiating cells. Some subpopulations, especially cancer stem cells and progenitor cells, originate from epithelial cancer cells of the primary tumor undergoing epithelial-mesenchymal transition (EMT) program [6, 26]. EMT is a crucial event in the dissemination of epithelial malignancies. Carcinoma epithelial cells are linked to each other and to basal membranes by protein junctions, which are abolished when cells aquire a migratory mesenchymal phenotype. This process is regulated by pleiotropic cytokines such as TGF-β acting through the "cadhedrin switch" [5]. Cell adhesion proteins are transcriptionally repressed by classical EMT-related signaling pathways. It is considered that Twist transcriptionally up-regulates Act-2 in breast cancer cells leading to increase migration and invasion [7]. Epithelial- mesenchymal transition is associated with acquisition of stem-like characteristics, connected

with their virtue of relative resistance to classical therapies, including radiation and chemotherapy. Bmi1, a protein promoting and maintaining self-renewal, is a direct transcriptional target of the EMT inducer Twist1 [23]. Moreover, many studies indicated ALDH1, being responsible for oxidation of retinol to retinoic acid, as a potent marker of breast cancer stem cell [13]. It was known that in breast cancer CD44+/CD24- cells are highly tumorigenic in immunodeficient mice. The mentioned markers are useful for identification of CTC with EMT and/or stemness phenotypes [1]. This observation was confirmed in women with metastatic breast cancer, where the population of CTCs was found to be highly enriched for cells that expressed the breast CSC markers CD44+/CD24- or aldehyde dehydrogenase (ALDH1).

Interestingly, micrometastasis detected in the bone marrow of these patients showed similar CSC profile, which suggests an important functional role of circulating tumor stem cells (CTSCs) in mediating micrometastasis[4].

DETECTION

The longstanding interest in CTCs has increased dramatically because of recent technological advances enabling their detection and isolation from peripheral blood of the patients with multiple different malignancies of solid organs as well as their capture and characterization. The most widely used method is CellSearch (Veridex, Raritan, NJ), which remains the only test approved by FDA. It relies on immunomagnetic capture of CTCs using antibodies against the epithelial cell adhesion molecule (EpCAM), which is expressed on the cell surface of many epithelial malignancies. This procedure is followed by additional staining with 4',6-diamidino-2-phenylindole-DAPI (to demonstrate that the detected event is a nucleated cell), and by immunofluorescence analysis with antibodies against cytokeratin (to show it is epithelial) and CD45 (to prove it is not a leukocyte) [18]. The EpCAM-expressing cells are automatically displayed visually and manually scored by a trained technologist. The results are reported as the number of cells meeting the definition per 7,5 mL of blood. Numerous studies have shown that the presence of elevated CTC levels, as demonstrated by CellSearch, is negatively correlated with prognosis in metastatic cancers of the breast, prostate and colon [8, 9, 12]. However, the clinical utility of monitoring CTC levels is still considered controversial. The American Society of Clinical Oncology (ASCO) Tumor Marker Guidelines Committee has not recommended incorporation of CTC levels by any method into standard care of patients with metastatic breast cancer [16]. The recent studies have suggested that the CellSearch technique may underestimate the number of EpCAM-expressing cells. Additionally, the use of any assay for CTCs in early-stage breast cancer can be predominantly limited by low sensitivity and poor specificity in this setting [10].

Among immunoselection-based technologies we also distinguish: Micro-Posts and Herringbone CTC Chips and flow cytometric methods. There are also functional assays (e.g. Epithelial ImmunoSPOT, collagen adhesion matrix), size and biophysical-based assays (like dielectrophoretic arrays, microfiltration devices, microfluidics separation). Reverse Transcriptase-Polymerase Chain Reaction is a commonly used procedure representing the last group of methods connected with genomic, transcriptional or translational factors. RT-PCR assays have been long used to detect low levels of cancer-specific mRNA in the mononuclear cell fraction of blood that are presumed to belong to CTC [10]. This strategy has been applied in breast, lung, prostate and colon cancers and has been found to be more sensitive than immune-capture techniques [15, 21]. However, none of the assays are ideal; they may lack sensitivity, technical and biological specificity. Technical non-specificity implies the detection of an entity that, after more careful scrutiny, appears not to be a cancer cell. Biologic non-specificity means that a test discovers an entity that by all criteria is a cancer cell, but which is unable to invade, proliferate and cause metastasis [19]. Therefore, not only enumeration, but also characterization of CTC should be beyond the scope of further research.

CLINICAL UTILITY

Predictive and prognostic capability of CTC has been tested in multitude of studies. Most of CTC investigations have been conducted in metastatic breast cancer. The first large, multi-institution, double blind prospective clinical trial evaluated the prognostic capability of CTCs in 177 patients with metastatic, measurable breast cancer. The level of CTC was assessed prior to beginning a new palliative treatment regimen for progressive disease, followed by repeat enumeration at first follow-up visit approximately 4 weeks later [9]. This landmark trial prospectively identified a CTC cut-off level of ≥5 cells per 7.5 ml of blood to be a reliable indicator of patients at higher risk of progression and decreased survival from metastatic breast cancer. The patients with <5 CTCs at baseline, and more importantly, at first follow-up after beginning a new treatment regimen had superior progression free and overall survival (PFS and OS), regardless of histology, hormone receptor and HER2 status [17]. A similar analysis of the prognostic value of CTCs among 185 newly diagnosed metastatic breast cancer patients prior to beginning first-line salvage therapy was performed in a large, retrospective, single-institution study [11]. The obtained results were close to these previously mentioned. Patients with ≥5 CTCs at baseline had a greater than three-and-a-half fold increased risk of death compared to those with <5 CTCs. The prognostic significance of CTCs was also independent of treatment choice, hormone receptor and HER2 status. Moreover,

greater than half of patients had bone as their first site of metastatic disease. The feature of bone metastasis was, upon multivariate analysis, an additional risk factor of death among patients with ≥5CTCs. In another large, retrospective, single-institution piece of research the correlation between the level of CTCs and histologic classification of tumor was analyzed in a cohort of 517 metastatic breast cancer patients with measurable disease prior to commencement of new palliative treatment. The observations showed that lobular histology and bony (but not visceral) disease burden were associated with higher number of circulating tumor cells [14]. All the mentioned trials used the CellSearch System (Veridex) as a method of CTC measurement.

Very few studies have been devoted to CTC in primary breast cancer. One of the latest investigations aimed to detect and characterize CTCs from peripheral blood of 61 patients with newly diagnosed breast cancer and absence of bone, visceral and cerebral metastasis before any treatment. Axillary lymph node invasion was assessed [5]. The authors reported to detect three main phenotypes of dedifferentiated CTCs (ddCTCs). The first phenotype was essentially mesenchymal with markers of EMT (PI3Kα, Akt-2 and Twist1), the second was characterized by a stemness marker- ALDH1 expression. The third phenotype was a mixture of the two previous ones. Therefore, the authors stated that if CTC were considered as a prognostic factor, the detection of all cell phenotypes should be taken into account. It is said that majority of ddCTCs with EMT and stemness characteristics are often not detected by generally used technologies in clinical trials. The demonstration of EMT program activation, leading to invasive properties and stemness of cancer cells, seems to be a new way to characterize CTC. The researchers conclude that the disease status should be analysed by the quantitative measurement of EMT gene expression. It could be efficient to control new drugs targeting mesenchymal cells. Eradication of ddCTCs would be the proof of therapeutic effectiveness in clinical trials. Their results also stressed that absence of lymph node invasion was not a criteria of non-dissemination, as blood is a major dissemination route.

SUMMARY AND FUTURE DIRECTIONS

The data demonstrated that CTCs occur both in early and metastatic breast cancer. Despite major advances in our understanding of cancer biology, we still lack detailed insight into the mechanisms of tumor establishment and metastatic spread. It seems predominantly essential to focus on assessment of cells showing mesenchymal or stemness characteristics, connected with epithelial to mesenchymal transition (EMT), as they are associated with enhanced ability of intravasation and dissemination. Targeting these types of cells would provide more clinically relevant prognosis

and predictive information than simple CTC counting. Their analysis should help in a more tight follow-up of the disease progression and might facilitate the evaluation of new drugs in clinical trials. In sum, it was stated that elevated CTCs were an ominous prognostic and predictive indicator, especially in metastatic breast cancer and a marker of increased morbidity that ultimately impacts mortality. On the contrary, the elimination or decrease of CTCs following treatment is connected with improved clinical outcomes. The ability to repeatedly sample an accessible tumor population such as CTCs may influence the optimal selection of therapies, based on confirmed target delivery. Therefore, it is crucial to develop additional technologies facilitating the reliable genomic and proteomic characterization of these cells to provide a profile of an individual patient's tumor. The full extend of CTCs utility has not been fully explored so far and needs further thorough scientific attempts to solve the encountered problems.

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