3 Disseminated Tumor Cells and Dormancy in Breast Cancer Progression

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Abstract

Hematogenous dissemination of single cancer cells is a common phenomenon in patients with solid tumors. These cells may experience different fates: most will die during the process; some will grow into metastasis and some will persist in secondary homing sites for many years in a state referred to as dormancy. The mechanisms of this state are still not clear; single cancer cells can survive either by completely withdrawing from the cell cycle or by continuing to proliferate at a slow rate that is counterbalanced by cell death. Another hypothesis assumes that at least some of dormant tumor cells feature stem cell-like characteristics that may contribute to their extremely long half-lives and enhance chemotherapy resistance. Breast cancer is particularly known for prolonged periods of clinical freedom of disease (sometimes up to 20–30 years), followed by a distant relapse. In this chapter, we explore the relationship between the clinical phenomenon of tumor dormancy and the disseminated tumor cells and discuss the potential implications for treatment.

Keywords

Breast cancer · Disseminated tumor cells (DTCs) · Circulating tumor cells (CTCs) · Cancer dormancy · Angiogenesis · Microenvironment · Metastasis

3.1 Introduction

Breast cancer (BC) is the most commonly diagnosed cancer and the second leading cause of death due to malignant disease in women worldwide. Despite adequate surgical and (neo) adjuvant systemic treatment, approximately one out of three to one out of four patients develops a relapse over time [1], suggesting that single tumor cells or tumor cell clusters, sometimes referred to as minimal residual disease (MRD), may survive at secondary sites and lead to tumor growth several years later [2]. The theory of hematogenous spread of solid tumors has been introduced by several researchers as early as nineteenth century, based on autopsy studies and the detection of cancer cells similar to those from the primary tumor in the blood [3, 4]. In the late twentieth century, the MRD research focused mostly on tumor cells found in the bone marrow. These disseminated tumor cells (DTCs) can be routinely detected in up to 40% of patients with primary BC and their presence predicts shorter

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disease-free and overall survival [5]. Further, a subset of these DTCs have been shown to survive chemotherapy; their persistence is associated with impaired clinical outcome as well [6, 7].

The development of improved assays for single cell detection and the introduction of new enrichment methods have enabled the research focus to shift to peripheral blood as an alternative compartment hosting tumor cells. The major advantage over bone marrow is the easy accessibility without the necessity of an invasive procedure and the possibility of serial measurements. When encountered in the blood, cancer cells are usually referred to as circulating tumor cells (CTCs). Currently, the overwhelming majority of studies registered in the ClinicalTrials.gov and EudraCT registries focuses on the CTCs in the blood; only six studies concern DTCs in the bone marrow [8]. With regard to their clinical relevance, CTCs have been shown to predict worse survival in both metastatic and early BC in large meta-analyses [9, 10].

In this chapter, we will discuss the role of DTCs in cancer dormancy and the clinical implications of this phenomenon.

3.2 Cancer Dormancy

Tumor dormancy, a phenomenon well-known to clinical oncologists, refers to a period of time in which tumor cells are assumed to be present but disease progression is not clinically apparent. BC is one of the entities known for prolonged asymptomatic periods, sometimes as long as 20–25 years, followed by a recurrence [11, 12]. About 20% of clinically disease-free breast cancer patients suffer from a relapse 7–25 years after mastectomy. Between 10 and 20 years after surgery, the rate of recurrence is relatively steady at about 1% per year [11, 13]. Similar courses of disease have also been observed in melanoma, prostate, thyroid and renal carcinoma, while late relapses are comparatively rare in colon and lung carcinoma [14]. As dormant single cells or micrometastases at secondary homing sites are widely assumed to be precursors of disease progression, their detection and possible elimination with adjuvant targeted therapies is a major goal of care of BC survivors.

Late recurrences might be due to the ability of DTCs to survive in a dormant state, evade therapies and finally transition to a proliferative state. Indeed, Meng et al. were able to detect single tumor cells in the blood in 36% of asymptomatic and clinically disease-free BC patients 7–22 years after diagnosis [15]. Recently, two large trials prospectively investigated the clinical relevance of CTC persistence. Sparano et al. showed that 4.8% of patients with non-metastatic BC had at least one CTC/7.5 ml blood around 5 years after diagnosis; these patients had a risk of relapse that was 18 times higher than that of CTC-negative women [16, 17]. Interestingly, CTC status was the strongest predictor of disease recurrence in the multivariate analysis. Similar results were reported in the German SUCCESS A trial [18]. In patients with hormone receptor positive BC, the CTC status 5 years after diagnosis significantly predicted shorter disease-free survival.

Yet, despite DTCs/CTCs being an independent prognostic predictor, the majority of patients with minimal residual disease does not develop metastases [5]. One possible explanation might be the phenomenon called "metastatic inefficiency". Although large numbers of cancer cells enter blood circulation every day [19, 20], most are already apoptotic or dead and it is currently assumed that less than one cell out of a thousand might give rise to subsequent secondary growth [21, 22]. Possibly, a significant proportion of viable tumor cells might be eliminated after entering blood vessels by shear mechanical forces of the blood stream [19, 23, 24].

There are currently no markers available to exactly predict the risk for late recurrence. Furthermore, it is not possible to predict which dormant tumor cells or micrometastases will eventually grow and which will stay dormant without ever becoming clinically relevant.

3.3 Potential Mechanisms and Clinical Relevance of Tumor Cell Dormancy

Despite major advances in therapy of BC leading to improvements in relapse-free and overall survival, a population of tumor cells is able to

survive systemic chemotherapy or targeted therapies and persist in blood or secondary sites. Cytotoxic treatment regimens especially target highly proliferative cells. In contrast, dormant tumor cells are mostly either slowly proliferating or remain in a state of quiescence, which is determined by the lack of proliferating markers (Ki-67, PCNA) accompanied by the lack of apoptotic markers (TUNEL, M30) and may explain the failure of conventional chemotherapy in some BC patients [25]. DTC dormancy was recently supported by DTCs expressing markers including NR2F1, DEC2 and p27 [26]. Of these, NR2F1 (Nuclear Receptor Subfamily 2 Group F Member 1) has been shown to play a key role in dormancy signaling [27]. DEC2 (also known as SHARP1 or BHLHE41) is a metastasis suppressor and is assumed to induce dormancy by activating p27 [28]. Moreover, current findings indicate that a subset of DTCs in breast cancer patients undergoes an epithelial-to-mesenchymal transition (EMT) and obtain a stem cell-like phenotype. DTCs that hold a stem cell-like phenotype (e.g. expression of ALDH, presence of CD44 and absence of CD24) are called cancer stem cells (CSCs) [29, 30]. A stem cell-like phenotype might be responsible for their resistance to cytostatic therapy [6, 31]. New treatment strategies that emerge from understanding the biology of dormant tumor cells include the ability to induce or maintain dormancy and induce the programmed cell death. Based on current dormancy studies, potential therapeutic strategies include: altering the microenvironment, targeting angiogenesis, targeting signal transduction and activating the immune system.

3.3.1 Microenvironment

Several clinical and pre-clinical studies have provided ample evidence that not only the cancer itself but also the tumor microenvironment plays a significant role in BC progression, metastasis and therapeutic outcome. Cancer cells are surrounded by various other cells with which they stay in constant interaction. The tumor microenvironment (TME) comprises of cancer cells, cancer associated fibroblasts (CAFs), endothelial cells and pericytes, immune and inflammatory cells, bone marrow derived cells and the extracellular matrix [32, 33]. The bidirectional cross-talk between cancer cells and the TME determines the extent of cell proliferation, angiogenesis, invasion and survival. Systemic treatment should therefore not only target cancer cells but also the surrounding TME. Treatment options are bisphosphonates (BPs) or the RANKL inhibitor denosumab, which are potent inhibitors of osteoclast-mediated bone resorption. Beyond their traditional use in bone metastatic disease, in vitro as well in vivo studies support a possible role as anticancer therapies by preventing cancer cell migration, and by promoting cancer cell death by changing the bone into a "hostile" environment. BPs and denosumab influence the TME by altered secretion of growth factors as well as cytokines and may act indirectly on cancer cells through microenvironmental changes using immunomodulatory and antiangiogenetic effects. Several studies confirmed the efficacy of BPs in preventing new bony and visceral metastases and their positive impact on progression-free and overall survival in selected BC patient subgroups (ABCSG-12, ZO-FAST, AZURE, NSABP B-34 trial) [34–38]. Small pilot studies have already demonstrated that BPs contributed to eliminate dormant DTCs, even after years of first diagnosis [39–42]. Moreover, the DTC status might be predictive of the efficacy of bisphosphonate therapy [43]. A current non-randomized phase II pilot study is evaluating the impact of denosumab on DTCs in patients with primary BC (NCT01545648). Patients with persistent DTCs received denosumab monthly for 6 months, then every 3 months for a total of 1-year treatment. To date, there are no published results yet.

While hypoxia is a poor-prognosis microenvironmental feature of solid tumors, it also seems to play an important role in tumor cell dormancy. One of the early responses to oxygen deficit is the reduction of oxygen consumption, achieved by decreased proliferation allowing cells to stay viable for long periods of time while dividing very slowly [44]. Primary tumors exposed to hypoxic

microenvironments have been shown to upregulate both hypoxia and dormancy genes. Interestingly, once cancer cells left the primary tumor, the expression of dormancy markers persisted, but the hypoxic response did not, suggesting that the dormancy-like response lasts longer than the hypoxic program [26]. Cell line-based studies have also demonstrated that repeated hypoxia leads to development of breast cancer cells adapted to hypoxic state by entering a dormant state [45].

3.3.2 Angiogenesis

Angiogenic dormancy can be defined as the state in which tumor cell proliferation is counterbalanced by apoptosis owing to poor vascularization. The lack of tumor angiogenesis impedes tumor growth beyond a microscopic size (2-3mm), resulting in an asymptomatic and non-metastatic state [46]. The angiogenic switch of cancer cells from a dormant, non-angiogenic phenotype to an active, angiogenic phenotype is a critical step and essential to promote fast-growing and expansion of tumor masses. Angiogenesis is therefore a critical feature of tumor growth and inhibition a potential treatment method. There are many growth factors involved in the physiological regulation of blood vessel formation. Blockade of even a single growth factor might limit vascular growth, with the most compelling evidence to date supporting blockade of VEGF. Several clinical trials on bevacizumab, a monoclonal antibody against VEGF, have shown improved progression-free survival when administered in combination with chemotherapy in the metastatic setting (E2100, RIBBON-1, AVADO) [47–49]. However, the overall survival was not affected. In early breast cancer, clinical studies on bevacizumab did not demonstrate a disease-free or overall survival benefit (ARTemis, GeparQuinto trial) [50, 51]. Besides bevacizumab, small inhibitors of VEGFR receptor tyrosine kinases (sunitinib) either alone or in combination with chemotherapy showed no clinical benefit for patients with advanced breast cancer [52]. Future trials might help to clarify whether prevention of the angiogenic switch with antiangiogenic agents might achieve clinically relevant results in terms of elimination of dormant tumor cells.

3.3.3 Targeting Signaling Pathways

Once dormant tumor cells leave their quiescent state, they may express specific receptors which, when activated can initiate downstream signaling resulting in the expression of genes for cancer cell proliferation, growth, survival, migration, and other vital cell cycle pathways. There is an increasing amount of targeted therapies which interfere with the function of specific molecules responsible for tumorigenesis and cell cycle.

The human epidermal growth factor receptor 2 (HER2) is one of the main targets. Several studies revealed that HER2 expression on both DTCs and CTCs differed from HER2 expression of the primary tumor and HER2 expression on DTCs and CTCs was correlated with poor prognosis [53–60]. During disease progression, HER2 gene amplification can be acquired even if the primary tumor was negative for HER2. Based on these observations, two pilot studies showed that adjuvant trastuzumab treatment is able to eliminate DTCs and CTCs [61, 62]. Yet, the recently published randomized TREAT CTC trial and the NSABP-B47 trial both failed to confirm the hypothesis that adjuvant trastuzumab can benefit women with HER2 non-amplified early breast cancer [63, 64].

The expression of the estrogen receptor (ER) on cancer cells is another main factor because endocrine adjuvant therapy remains a cornerstone of breast cancer treatment. In line to HER2, several studies have revealed a discordance of ER status between primary tumor and DTCs as well as CTCs [60, 65, 66]. This might be relevant for clinicians when selecting patients for adjuvant endocrine therapy. A loss of ER-positivity of MRD might explain the failure of adjuvant endocrine therapies in a subgroup of ER-positive BC patients. Moreover, the discordance could be important for patients lacking ER on the primary tumor but showing ER-positive DTCs/CTCs

because they might benefit from an endocrine therapy. Determining the phenotype of DTCs and CTCs is therefore becoming more and more important, as occult tumor cells are the targets of all adjuvant treatment regimes. Besides local treatment of the primary tumor and lymph node metastases, the definitive success of BC therapy is dependent on the ability to eliminate residual cancer cells which are persistent after primary surgery, before they become clinically evident.

There are increasing numbers of other specific agents targeting the signal transduction, including everolimus (mTOR inhibitor), lapatinib (EGFR and HER2 inhibitor), pyrotinib (HER1, HER2, and HER4 inhibitor), pertuzumab (HER2 dimerization inhibitor), ribociclib/abemaciclib/palbociclib (cyclin-dependent kinase 4/6 inhibitors), T-DM1 (combination of trastuzumab and the chemotherapy medicine emtansine) and alpelisib (an α-specific PI3K inhibitor). The ability to determine and monitor the biology of MRD cells and to follow changes on proteomic, transcriptomic and genomic level in real-time may allow the tailoring of conventional medical treatment to individual characteristics. However, clinical studies demonstrated that elimination of dormant tumor cells may not directly impact the survival. Prospective randomized controlled trials are therefore needed to investigate whether patients with persistent MRD benefit from these agents.

3.3.4 Immune System

The inherent capacity of the immune system has a major impact on the balance between dormant tumor cells and tumor growth. The dynamic process consisting of immunosurveillance and tumor progression, referred to as i*mmunoediting, is made up of three phases*: elimination, equilibrium, and escape [67]. In the equilibrium phase, the immune system holds tumor cells in a state of functional dormancy or quiescence by host-derived cytotoxic T lymphocytes [68]. Various approaches have been developed to sustain such endogenous host-protective immune responses including immunomodulating antibodies which specifically block immune checkpoint inhibitors and potentially expand endogenous anticancer immune responses. Most promising immunomodulating antibodies are monoclonal anti-PD-1 (pembrolizumab) or anti-PD-L1 (atezolizumab, durvalumab) antibodies for the treatment of patients with advanced triple negative breast cancer. Clinical trials showed objective response rates in the 5%–19% range [69–71]. Host-protective immune responses can be also amplified by vaccines, which boost naturally occurring antitumor immune responses. Many different types of cancer vaccines have been constructed from distinct immunogenic sources represented by whole tumor lysates, tumor antigenic peptides, DNA, RNA, and viruses. Moreover, they can be combined with immunoadjuvants, which contribute to the immune stimulation. Encouraging results are coming out during several clinical phase II/III trials. NeuVax, AVX901, and INO-1400 are currently the most promising BC vaccines [72]. In (dormant) MRD, favorable effector-target ratios prevail and therefore might be optimally suited for vaccines and immunotherapy with antibodies.

3.4 Conclusions

Tumor dormancy is a clinically relevant phenomenon that reflects the ability of minimal residual disease to elude systemic therapy and persist as single cancer cells or micrometastasis at secondary homing sites. Dormant cells can either completely withdraw from cell cycle and remain in mitotic arrest or divide at a very slow rate counterbalanced by cell death. However, the exact mechanisms underlying tumor dormancy and leading to activation of dormant cells are still unclear. Possibly, angiogenetic and immunomodulatory factors contribute to the development of a microenvironment most suitable for hosting dormant cells. To effectively target these cells, better understanding of tumor dormancy is necessary and might help to design new targeted approaches to control this step of disease progression.

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