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Review

Clinical and molecular complexity of breast cancer metastases

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ABSTRACT

Clinical oncology is advancing toward a more personalized treatment orientation, making the need to understand the biology of metastasis increasingly acute. Dissecting the complex molecular, genetic and clinical phenotypes underlying the processes involved in the development of metastatic disease, which remains the principal cause of cancer-related deaths, could lead to the identification of more effective prognostication and targeted approaches to prevent and treat metastases. The past decade has witnessed significant progress in the field of cancer metastasis research. Clinical and technological milestones have been reached which have tremendously enriched our understanding of the complex pathways undertaken by primary tumors to progress into lethal metastases and how some of these processes might be amenable to therapy. The aim of this review article is to highlight the recent advances toward unraveling the clinical and molecular complexity of breast cancer metastases. We focus on genes mediating breast cancer metastases and organ-specific tropism, and discuss gene signatures for prediction of metastatic disease. The challenges of translating this information into clinically applicable tools for improving the prognostication of the metastatic potential of a primary breast tumor, as well as for therapeutic interventions against latent and active metastatic disease are addressed.

1. Introduction

Tumor metastasis is a major clinical challenge accounting for the vast majority of cancer related deaths. Although only 5–10% of newly diagnosed breast cancer patients present with cancer that has metastasized to distant body parts [1–3], the risk of developing metastatic disease in patients with localized primary disease following successful primary tumor resection and adjuvant therapy remains high. It is estimated that up to 30% of node-negative breast cancer patients and an even larger fraction of patients with node-positive disease will develop metastatic disease despite receiving standard treatment [1,4]. These figures and the fact that distant recurrent disease must generally be viewed as an incurable disease indicate the high clinical burden of metastatic breast cancer (MBC) and underscore the urgent demand for better strategies for clinical intervention for those more than half a million women world-wide still succumbing to this disease annually [5].

It has been recognized for some time that breast cancer dissemination is a non-random, organotropic process, originally based on Paget's theory of "seed and soil" [6]. Factors influencing the development and localization of breast cancer metastases have been identified and will be discussed in this review. Furthermore, important associations between molecular subtypes and risk as well as site/s of recurrence have emerged and will be reviewed herein. Challenges in the path to clinical translation and how recent advances in the understanding of the complexity of breast cancer metastases may inform future management of early stage breast cancer patients are addressed.

2. Tumor progression

Tumor progression from an early pre-neoplastic lesion through invasive cancer to the development of clinically detectable distant metastases may be conceived as an evolutionary process, involving multiple genetic and epigenetic alterations affecting both tumor cells and the surrounding stroma, allowing seeding of metastases at distant sites. Although the path toward metastatic colonization

Abbreviations: BBB, blood–brain barrier; BRCA, breast cancer associated; ER, estrogen receptor; CTC, circulating tumor cell; ctDNA, circulating tumor DNA; DRFI, distant recurrence-free interval; DTC, disseminated tumor cell; EMT, epithelial-to-mesenchymal transition; HER2, human epidermal growth factor receptor 2; MBC, metastatic breast cancer; PR, progesterone receptor; TNBC, triple-negative breast cancer.

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is a complex and multi-faceted process, it is also thought to be highly inefficient. The likelihood of a circulating tumor cell forming a metastatic colony in a distant organ is in fact extremely low. Most cells that leave a tumor die, often due to inability to infiltrate distant organs [7,8]. Data from preclinical animal studies have shown that less than 0.02% of circulating tumor cells can survive and have the capability to seed metastases [9]. To develop metastases, primary tumor cells must invade and escape from the complex physical barriers (extracellular matrix, basement membrane and vasculature) at the primary site, intravasate into the lymphatic or vascular system, exit it to infiltrate distant organs and continue to proliferate in this foreign milieu [10]. In this context, there exists considerable heterogeneity in the metastatic potential of individual cells within the bulk of a primary tumor [11–15].

The metastatic propensity of a tumor cell is thought to be influenced by both the cell of origin and the oncogenic alterations present in the tumor. For example, the same oncogenic mutations occurring in cells at different stages of differentiation or lineages (*e.g.* stem cells) may hence lead to distinct metastatic propensities [10,16,17]. In addition, the type of oncogenic driver mutation may also influence the ability of a tumor to metastasize [17].

2.1. Linear progression model

The question of when and how metastases spread is complex and has multiple answers. Tumor cells can adopt different evolutionary paths to seed metastases and these paths may vary within and between different tumors. Two classical models of tumor metastasis are widely acknowledged. Traditionally, it has been considered that metastatic dissemination is a "late" event, occurring when the primary tumor is large [9]. In this linear progression model, heterogeneous clones in the primary tumor undergo a sequential clonal selection process, during which subclones with metastatic propensity are selected for and undergo further mutational changes endowing them with survival advantages and the capacity to grow as overt metastases in different organs [14,16,18]. Indeed, primary tumor size is a risk factor for metastatic progression, providing indirect support for this model [19]. Moreover, early studies reporting similar gene expression signatures between metastases and their corresponding primary tumors can be interpreted as further support [20]. This concept constitutes the theoretical basis for early detection, *e.g.* mammography screening, as a tool to reduce metastatic disease. In contrast, as reported in other studies [21,22], primary tumors may already contain a gene expression profile that is strongly predictive of metastasis and poor survival, thus challenging the notion that metastatic ability is acquired late during tumor progression. Given the wide degree of intra-tumor heterogeneity, analyzing a single small biopsy from a tumor may underestimate the complexity of the molecular landscape. This factor is a limitation of most genetic studies performed so far and presents a major challenge to the interpretation of these correlations as well as to the successful development of precision medicine [23].

2.2. Parallel progression model

The parallel progression model postulates that the metastatic potential is acquired very early in disease progression, when the primary lesion is small or even undetectable. It is based on the notion that disseminated cells evolve independently of the primary tumor and that different tumor clones can be seeded in parallel to distant sites [24,25]. This model implies that cancer is a systemic disease, requiring systemic (adjuvant) treatment at an early stage for efficient eradication [25,26]. In support of this model are observations demonstrating significant genetic differences between paired primary breast cancers and lymph node metastases [27–29], as well as discordances between primary tumors and distant relapses when conventional prognostic markers (ER, PR or HER2) are assessed [30,31]. In the study by Falck et al. [30], no significant discordance in single biomarkers was observed between primary tumors and synchronous lymph node metastases. However, by combining individual biomarkers to classify tumors into molecular subtypes according to the St Gallen guidelines [32], significant discordances in molecular subtypes were revealed between the primary tumors and lymph node metastases, and the prognosis was strongly correlated with the subtype of the metastatic lymph node. Moreover, an inferior outcome has also been reported when the phenotype differed between primary and metastatic disease [33–37], suggesting that fundamental alterations in the course of dissemination occur, thereby affecting outcome.

The detection and prognostic relevance of circulating tumor cells (CTCs) in patients with metastatic breast cancer as well as in patients with early-stage disease [38,39] lends additional evidence that parallel progression may occur. Nevertheless, most metastases are generally detected years, or even decades following diagnosis and treatment of the primary tumor. From this perspective, CTCs, disseminated tumor cells (DTCs) in the bone marrow or even circulating cell free tumor DNA (ctDNA) may be more relevant for the purposes of predicting disease progression and monitoring response to treatment [40]. As such, several clinical studies have been initiated to develop and validate their potential to serve as powerful tools for non-invasive detection of early/late metastatic disease and biomarkers for response to therapy.

Irrespective of the route of progression favored by a specific tumor, it is still unclear if each metastasis originates from a single progenitor cell (monoclonal seeding) [14,18], or if polyclonal seeding, where some metastases may originate from multiple events involving a heterogeneous mix of distinct sub-clones from the primary tumor as well as clones from other metastases [41–43] is an alternate path. Gundem et al. recently performed whole genome sequencing of serial primary tumors and metastases from patients with metastatic prostate cancer and confirmed that metastases from different organ sites in the same patient had sub-clonal alterations originating from multiple distinct clones, some of which were also found in the primary tumor, suggesting that this polyclonal seeding must have arisen both from the primary tumor and from other metastases [41–43]. Regardless of the mode of progression or the origin of metastatic cancer cells, considerable advancements in the knowledge of the molecular events underlying the development of metastatic disease are required before successful treatment and prevention become a reality.

3. Genes mediating breast cancer metastasis

While many of the transforming genetic and epigenetic changes necessary for oncogenesis are also necessary for metastatic progression, the principal steps of the metastatic cascade are accomplished by four main categories of genes (reviewed in detail elsewhere [16,17,44]). Briefly, the first group, *metastasis initiation genes*, allow aggressive cells to invade the surrounding tissue, attract a supportive stroma, facilitate the dispersion of cancer cells and may also play a role in infiltrating distant metastatic niches. Several genes involved in epithelial-to-mesenchymal transition (EMT; *e.g. TWIST1*, *SNAI1*, *SNAI2*) [10,16,17,44,45], extracellular matrix degradation (matrix metalloproteinases, MMPs), hypoxia (*e.g. HIF1A*), and angiogenesis (*VEGF*) have been associated with this step. The expression of these metastasis initiation genes and their target genes in primary tumors is prognostic of poor outcome [16].

Metastasis progression genes comprise the second category and co-operate to provide tumor cells with specialized

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