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Review

How to study and overcome tumor heterogeneity with circulating biomarkers: The breast cancer case

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ABSTRACT

Breast cancer ranks first among female cancer-related deaths in Western countries. As the primary tumor can often be controlled by surgical resection, the survival of women with breast cancer is closely linked to the incidence of distant metastases. Molecular screening by next generation sequencing highlighted the spatial and temporal heterogeneity of solid tumors as well as the clonal evolution of cancer cells during progression and under treatment pressure. Such findings question whether an optimal assessment of disease progression and a screening for druggable mutations should be based on molecular features of primary or recurrent/metastatic lesions and therefore represent a crucial element for failure or success of personalized medicine. In fact, new targeted therapies may induce only short-term benefit annulled by the emergence of resistant clones with new driver mutations which would need to be rapidly and reliably identified. Serial tissue sampling is therefore essential but, unfortunately, also represents a problem since biopsies from solid lesions, which are invasive and potentially painful and risky, cannot be easily repeatedly sampled, are inaccessible or may not fully reflect tumor heterogeneity. The need to early detect and strike this "moving target" is now directing the scientific community toward liquid biopsy-based biomarkers, which include circulating tumor cells (CTC) and cell-free circulating tumor DNA (ctDNA), can be repeatedly assessed through non-invasive and easy-to-perform procedures and may act as reliable read-outs of functional and molecular features of recurrent/metastatic lesions. In this review we summarize the outcome of CTCs and ctDNA in breast cancer, with special reference on their role on unveiling and overcoming tumor heterogeneity, on their potential relevance for tumor surveillance and monitoring, and for the selection of therapeutic options. Finally, we propose integration between blood-based molecular and clinical approaches for monitoring disease progression according to the specific pattern of recurrence of the most aggressive breast cancer molecular subtypes.

1. Introduction

Breast cancer is the leading cancer in women worldwide, with an estimated incidence in 2012 of 494,100 and mortality of 142,980 among European women (http://globocan.iarc.fr). Although there have been significant advances in the clinical management over the past few decades, women continue to die due to this disease.

The effective and efficient management of cancer patients relies on early diagnosis, proper treatment and monitoring of response. Current methods for detection and monitoring of breast cancer progression, metastasis, and recurrences lack sensitivity and have not yet been proven to significantly extend overall survival (OS) [1]. Therefore, there is a clear clinical need for alternative diagnostic techniques that allow an earlier detection of metastasis enabling for an initiation of therapies in the presence of a smaller tumor burden, more likely to have fewer oncogenic events [2].

The development of tailored treatments is increasingly dependent on the understanding of tumor biology, and predictive biomarkers are

Abbreviations: ABC, advanced breast cancer; CGH, comparative genomic hybridization; CNA, copy number alterations; CTC, circulating tumor cells; ctDNA, cell-free tumor DNA; DEP, dielectrophoretic; DFS, disease-free survival; dPCR, digital PCR; EBC, early breast cancer; EGFR-2, epidermal growth factor receptor-2; ER, estrogen receptor; IF, immunofluorescence; ITOMIC, intensive trial of omics in cancer; LABC, locally advanced breast cancer; MBC, metastatic breast cancer; MIC, metastasis initiating cells; MPS, massively parallel sequencing; MRD, minimal residual disease; NCCN, National Comprehensive Cancer Network; NA, not applicable; NAC, neo-adjuvant chemotherapy; NGS, next generation sequencing; NL, Netherlands; NOS, not otherwise specified; OS, overall survival; PBMC, peripheral blood mononucleated cells; PFS, progression-free survival; PR, progesterone receptor; ptDNA, plasma tumor DNA; SBRT, stereotactic body radiotherapy; SNV, single nucleotide variant; WES, whole exome sequencing

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crucial in the field of personalized medicine as they enable definition of the patient population most likely to benefit from a specific therapy. The current gold standard of breast cancer diagnosis is the histological examination of tumor tissue, generally obtained either by biopsy or surgical excision of the primary tumor or of metastatic lesions. However, these procedures are invasive, often non repeatable over time, and may cause some risk to the patient.

The use of biological fluids such as blood as a source of non-invasive cell- and nucleic acid-biomarkers has recently raised interest in the oncology community. So-called "liquid biopsies" hold great clinical promise, as their non-invasive nature allows for rapid and repeated sampling – features that permit their use in screening programs – and for the close monitoring of treatment response and disease progression, enabling for earlier intervention and dynamic treatment management [3]. Furthermore, there is an increasing awareness of the genetic heterogeneity of tumors and a perception that tissue biopsies may miss this diversity [4]. Liquid biopsies in contrast can capture the entire genetic landscape of breast cancer and consequently have the potential to improve current treatment selection, allowing a personalized approach for each patient.

Although the majority of research on liquid biopsies to date has been centered on the isolation and characterization and downstream analysis of circulating tumor cells (CTCs), the focus of clinical studies is turning toward circulating cell-free tumor DNA (ctDNA), being easier to isolate and detect, and amenable to perform retrospective analyses with a high sensitivity readout. This review summarizes the outcome of cell- and DNA-based circulating biomarkers in breast cancer, with special emphasis on their role on unveiling and overcoming tumor heterogeneity, on their potential relevance for tumor surveillance and monitoring, and for the selection of therapeutic options. We report the emerging use of molecular information from ctDNA and CTCs in clinical research also considering practical factors such as clinical validation that seem to be limiting their integration into clinics practice, and the still unproven standardization of pre-analytical and analytical steps. Ultimately, in early breast cancers (EBC) we would suggest integration between blood-based molecular and clinical approaches for monitoring disease progression by shaping the timing of liquid biopsy collection according to the specific pattern of recurrence of the most aggressive intrinsic molecular subtypes.

2. The multiple faces of BC heterogeneity

Breast cancer heterogeneity has a tremendous impact on clinical management of the disease impinging on prognosis and posing fundamental challenges for treatment choice [5]. Besides inter-patient heterogeneity, well recognized by the different molecular subtypes, individual therapy responses and clinical outcomes, breast cancer is characterized by a broad spectrum of intra-tumor morphological and molecular diversity [4]. Pathologists have long recognized that breast tumor samples are morphologically heterogeneous comprising distinct cell subpopulations [6–9], with wide variation in the expression levels of Estrogen and Progesterone Receptors (ER, PR) and HER2 within different areas of the same tumor as well as between matched primary and metastatic lesions [10,11].

The recent development and implementation of advanced sequencing technologies and bioinformatic tools, while enabling a deep characterization of the genetic landscape of breast cancer lesions, have further revealed a high degree of genomic diversity within a single tumor lesion, across different regions (spatial heterogeneity), and over time (temporal heterogeneity) [12]. Indeed, geographical heterogeneity evolves over time with disease progression [13] and cancer cells, due to constant remodeling of their genome and clonal selection, acquire mutations conferring growth and invasive advantage. As a consequence, the analysis of an individual cancer sample may be regarded as only a sort of "snap-shot" in space and in time.

The coexistence of different cancer subclones harboring distinct somatic DNA alterations in breast tumor lesions, that is a direct evidence for intra-tumor heterogeneity, has been described by a number of studies using high-resolution microarray-based comparative genomic hybridization (CGH) [14–16] and more recently by Next Generation Sequencing (NGS) [17–19]. An approach based on multi-regional sampling and sequencing on a substantial series of breast cancers [19] allowed to infer subclonal structure of the primary lesions and demonstrated that subclonal diversification may affect relevant genes for breast cancer (including PIK3CA, TP53, PTEN, BRCA2, and MYC) and varied among cases without evidence of specific temporal order.

Comparative studies based on high-throughput molecular analyses (*i.e.*, high-resolution array CGH profiling and NGS) have also been performed to determine the concordance rate of mutations between primary tumors and paired metastases [20–25]. Globally, these studies provided evidence of high degree of similarity between the two entities, notwithstanding qualitative and/or quantitative divergence in their molecular profile. In particular, Goswami and colleagues [20] reported the presence of private mutations in the primary tumors rather than in the paired metastatic lesions, leading to two alternative scenarios: the metastases branched off before the acquisition of novel mutations within the primary tumor or the observed branched evolution could be attributed to the presence of intratumoral heterogeneity. High level of global concordance between primary and secondary tumors has been reported also in a recent study [25] even though the authors found some divergent mutations in actionable driver genes such as PIK3CA, TP53, and ERBB2. Moreover, very insightful information came from The progressive Intensive Trial of Omics in Cancer (ITOMIC) that enrolled patients with triple negative breast cancer (TNBC) with bone metastasis treated with cisplatin [26] for a comprehensive analysis of multiple biopsies collected over time for each patient (*i.e.*, at study beginning, at progression and at autopsy). ITOMIC revealed that tumor samples acquired genomic aberrations in response to each treatment cycle but also shared mutational features, thus indicating the presence of recurrent tumor cell populations that might be instrumental for the outgrowth of subclonal tumor cells in response to therapy. Since intra-patient and temporal heterogeneity may impair the response to specific targeted treatments, an optimal therapeutic strategy should include a comprehensive molecular analysis of multiple biopsies and should offer multi-targeted therapy regimens. This indication should be taken into account for future study design in the context of precision medicine in metastatic patients because gain or loss of even a single mutation may affect signal transduction pathways and compromise successful clinical development of molecularly targeted drugs. Indeed, in line with the current ASCO guidelines, which recommend the biopsy in patients with accessible metastases for retesting ER, PR, and HER2 [27], the information deriving from genomic profiling of primary and metastatic tumor samples should be integrated.

Improving our ability to identify which tumor subclone detected in the primary lesion is likely to become clinically relevant in terms of therapy resistance, risk of relapse, and metastatic dissemination represents a very crucial issue. To this aim, large scale clinical trials encompassing patients with tumors at different stages are paramount to establish the clinical value of spatial and temporal diversity in the genomic landscape of breast cancer to eventually guide treatment. However, several limitations affect genetic characterization of metastatic deposits as well as of primary tumor tissues: (i) single-tumor biopsy samples unlikely could encompass the genetic landscape of the entire tumor, which inevitably leads to an underestimation of the mutational load of heterogeneous lesions and thus to inaccurate prediction of proper treatment; (ii) multiregional and iterative tumor biopsies are unfeasible due to procedure invasiveness and associated risk of morbidity; (iii) obtaining good quality biopsies of metastatic lesions is frequently challenging and metastatic sites are often inaccessible.