frontiers in Medicine

REVIEW
published: 08 December 2017
doi: 10.3389/fmed.2017.00227

Tumor Heterogeneity in Breast Cancer

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Breast cancer is a heterogeneous disease and differs greatly among different patients (intertumor heterogeneity) and even within each individual tumor (intratumor heterogeneity). Clinical and morphologic intertumor heterogeneity is reflected by staging systems and histopathologic classification of breast cancer. Heterogeneity in the expression of established prognostic and predictive biomarkers, hormone receptors, and human epidermal growth factor receptor 2 oncoprotein is the basis for targeted treatment. Molecular classifications are indicators of genetic tumor heterogeneity, which is probed with multigene assays and can lead to improved stratification into low- and high-risk groups for personalized therapy. Intratumor heterogeneity occurs at the morphologic, genomic, transcriptomic, and proteomic levels, creating diagnostic and therapeutic challenges. Understanding the molecular and cellular mechanisms of tumor heterogeneity that are relevant to the development of treatment resistance is a major area of research. Despite the improved knowledge of the complex genetic and phenotypic features underpinning tumor heterogeneity, there has been only limited advancement in diagnostic, prognostic, or predictive strategies for breast cancer. The current guidelines for reporting of biomarkers aim to maximize patient eligibility for targeted therapy, but do not take into account intratumor heterogeneity. The molecular classification of breast cancer is not implemented in routine clinical practice. Additional studies and in-depth analysis are required to understand the clinical significance of rapidly accumulating data. This review highlights inter- and intratumor heterogeneity of breast carcinoma with special emphasis on pathologic findings, and provides insights into the clinical significance of molecular and cellular mechanisms of heterogeneity.

Keywords: breast cancer, tumor heterogeneity, histopathology, biomarkers, genetic markers

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pathology, a section of the journal Frontiers in Medicine

Received: *20 October 2017*
Accepted: *28 November 2017*
Published: *08 December 2017*

Citation:
Turashvili G and Brogi E (2017) Tumor Heterogeneity in Breast Cancer. Front. Med. 4:227. doi: 10.3389/fmed.2017.00227

INTRODUCTION

Tumor heterogeneity is one of the hallmarks of malignancy. Intertumor heterogeneity is observed in breast carcinomas from different individuals. Intratumor heterogeneity is due to the presence of heterogeneous cell populations within an individual tumor (1). Early reports defined tumor heterogeneity based on the identification of intratumor cell populations with different characteristics, including tumorigenicity, treatment resistance, and metastatic potential (2–4). Although the heterogeneity of breast cancer at the cellular level was recognized already in the nineteenth century (5), its clinical relevance was first established about 30 years ago, with the introduction of estrogen receptor (ER) testing (6). Variation in the expression of ER among different tumors or distinct cell populations within a single tumor was thought to account for differences in clinical behavior and

treatment response (6). Currently, understanding the molecular and cellular mechanisms of tumor heterogeneity that are relevant to the diagnosis, prognosis, and therapy of breast cancer is subject of intense research.

INTERTUMOR HETEROGENEITY

Clinical and Histopathologic Heterogeneity

Intertumor heterogeneity of breast cancer is best illustrated by clinical staging of the disease based on physical examination and imaging findings. The TNM staging system by the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) incorporates Tumor size, regional lymph Node status, and distant Metastases (7). Standard breast cancer treatment is based on the tumor characteristics, including clinical stage, histopathologic features, and biomarker profile, and is affected by the patient's age, menopausal status, and general health (8). The aforementioned traditional clinicopathologic variables have a profound impact on survival, and account for most of the differences in clinical outcome among patients with breast cancer (9).

The morphologic heterogeneity of breast carcinoma constitutes the basis for the histopathologic classification of breast cancer. Invasive ductal carcinoma (IDC) of no special type or not otherwise specified (NOS) is the most common (40–75%) histologic type of invasive breast cancer. Albeit common, IDC NOS is not at all well defined, and the 2012 World Health Organization (WHO) classification defines IDC NOS by exclusion, as "the heterogeneous group of tumors that fail to exhibit sufficient characteristics to achieve classification as a specific histological type" (9). In addition to IDC NOS, the WHO classification includes 21 special subtypes with distinctive morphologic features, of which invasive lobular carcinoma (ILC) is the most frequent (5–15%) (9). The other special subtypes of breast carcinoma are rare and differ significantly with regard to prognosis and response to adjuvant treatment (10–13). Tubular, mucinous, and papillary carcinomas usually have excellent clinical outcome compared to IDC and ILC (14, 15) and are not always treated with chemotherapy (16). By contrast, metaplastic carcinoma and poorly differentiated IDC NOS have a significantly worse outcome and are routinely treated with systemic chemotherapy (9).

The grade of breast carcinoma also highlights its tumor heterogeneity. Grade is assessed according to a 3-tier (low, intermediate, high) system based on the evaluation of three morphologic parameters, namely the percentage of the tumor arranged in glands and tubular structures, the degree of nuclear pleomorphism, and the mitotic rate (17). The grade of breast carcinoma is a robust prognostic factor, and is incorporated in clinical decision-making tools, such as the Nottingham Prognostic Index and Adjuvant! Online (9, 18). Breast cancers of different grades also show different profiles by proteomic, genomic and transcriptomic analysis (19–21). In multivariate models that include gene signatures, grade remains an independent prognostic factor for ER-positive tumors (22). Grade 1 and 3 breast carcinomas likely represent two very different diseases, and molecular data indicate that the progression from low- to high-grade carcinoma is exceedingly rare (9).

Biomarker Heterogeneity

The expression of ER, progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) is assessed routinely in all invasive breast carcinomas by immunohistochemistry (IHC) according to the recommendations by American Society of Clinical Oncology/College of American Pathologist (ASCO/CAP) (23, 24). The aforementioned biomarkers are established prognostic and predictive factors and their expression in breast carcinomas is critical in guiding patient treatment (8, 25).

Estrogen receptor and PR are expressed in approximately 80% and 60–70% of breast carcinomas, respectively (26, 27). Although ER-positive tumors co-express PR (ER+/PR+) in 70–80% of cases, some breast carcinomas are ER+/PR− or, rarely, ER−/PR+. The response to hormonal treatment also varies, with the best response (approximate rate of 60%) in ER+/PR+ tumors and lower rates in ER+/PR− and ER−/PR+ tumors (9).

The HER2 oncoprotein is overexpressed in approximately 15–20% of primary breast carcinoma as detected by IHC staining using the approved reagents, testing protocols, and scoring algorithm. Positive (3+) HER2 staining highly correlates with gene amplification (9); depending on the definition of HER2-equivocal (2+) staining, approximately 10–20% of HER2-equivocal breast carcinomas are found to be HER2-amplified by *in situ* hybridization (ISH). HER2-positive breast carcinomas have the most unfavorable prognosis of all types of invasive breast cancers, but they show high rate of response to anti-HER2 targeted therapy (e.g., trastuzumab, lapatinib) (28), as documented by the pathologic complete response post-neoadjuvant treatment in about 50–60% of patients with HER2-positive tumors (29).

Breast carcinomas that do not express ER, PR, and HER2, usually referred to as "triple-negative" breast carcinomas, constitute an extremely heterogeneous group histologically, genetically, prognostically as well as with regard to treatment response. Emerging data suggest that nuclear expression of the androgen receptor (AR) can be detected in 12–55% of triple-negative (ER-/PR-/HER2-) breast cancer (30–32). The prognostic significance of AR expression in triple-negative carcinomas is controversial, but it is associated with improved survival in other tumor subtypes (33). Ongoing clinical trials evaluating AR antagonists (such as bicalutamide and enzalutamide) in AR+ (defined as nuclear staining in ≥10% of tumor cells by IHC) triple-negative breast carcinomas show promising results (31, 34). AR positivity is associated with lower Ki-67 proliferation index, suggesting that AR may promote a stem-like or mesenchymal phenotype in this subset of tumors (32). No standardized assays or guidelines for evaluating the AR expression in breast carcinoma are available at present.

Hundreds of other biomarkers have been investigated in breast cancer for potential diagnostic, prognostic, and therapeutic implications. Functional classification of these biomarkers includes growth and proliferation (Ki-67, survivin, NGAL), invasion and metastasis (p53, MMP-9, SK1, DcR3, COX2, EZH2, microRNAs miR-105, and miR126), epithelial–mesenchymal transition (EMT) (WNT5A/B, Pea3), immune response (PD-L1), therapy

resistance (HER2Δ16, pSTS3, KLK10), survival (miR-574-3p, miR-660-5p, PIWIL3, PIWIL4), and many others (35). The magnitude of the effect of tumor heterogeneity on biomarker expression or its clinical significance remains uncertain. A systematic approach and standardized quantitative reporting of biomarkers is required to better guide therapeutic decisions.

Genetic Heterogeneity

Gene expression analysis classifies breast cancer into four major intrinsic molecular subtypes with prognostic and therapy implications: luminal A, luminal B, HER2-enriched, and basal-like (36). The luminal A and luminal B subtypes exemplify tumor heterogeneity within ER-positive breast carcinomas and have better survival than HER2-enriched and basal-like subtypes. Both luminal subtypes express ER, but the luminal B tumors are characterized by increased expression of proliferation-associated genes and have worse prognosis than luminal A tumors (37). The HER2-enriched subtype is characterized by increased expression of HER2 and proliferation genes and includes ER-/PR-/HER2+ and ER+/PR+/HER2+ tumors. The basal-like subtype is enriched for genes expressed in basal epithelial cells, and is triple-negative in 70% of cases (36). Additional subtypes include claudin-low tumors with stem-like signature (38) and AR-positive molecular apocrine tumors (39). Meta-analysis of gene expression studies suggests that the prognostic impact of different signatures is related to the proliferation-associated genes (40). Although gene expression profiles can predict response to chemotherapy and recurrence risk (41), classification of breast carcinoma based on gene expression is hindered by clinical and molecular heterogeneity. Patients with breast carcinoma of the same molecular subtype and receiving identical treatments may have different clinical outcomes and/or acquire resistance to therapy (42). Frequent (>10%) somatic mutations in TP53, PIK3CA, and GATA3 have been documented in breast carcinomas (43). More recent studies have yielded other molecular subgroups, including a molecular classification based on integrated genomic and transcriptomic profiling of 2,000 breast tumors yielding 10 novel subtypes of breast cancer with distinct clinical outcomes (44, 45). Additional studies are needed to evaluate the practical clinical relevance and treatment implications of driver-based breast cancer classifications.

RNA-based multigene expression assays have been developed to estimate recurrence risk in ER-positive and/or lymph node-negative patients. According to the ASCO clinical practice guidelines (8), some multigene expression assays show sufficient evidence for clinical utility. They include the 21-gene assay Oncotype DX (46), the 11-gene EndoPredict (47), the 50-gene assay Prosigna based on the prediction analysis of microarray 50 model (48–50), and the 7-gene based Breast Cancer Index (BCI) (51). Prosigna, BCI, and EndoPredict predict late recurrence and subclassify tumors into molecular subtypes (52). Oncotype Dx is a reverse transcriptase polymerase chain reaction-based assay, and quantifies the likelihood of early distant recurrence and chemotherapy benefit for patients with lymph node-negative, hormone receptor-positive, HER2-negative breast cancer (46, 53). The risk of recurrence is expressed as a numerical value between 0 and 100, referred to as recurrence score (RS). Tumors are stratified into low risk (RS $\leq$ 17), intermediate risk (RS 18–30), and high risk (RS $\geq$ 31) categories (46). In patients with tumors of RS $\leq$ 17, the benefit of chemotherapy is quantified as too small (2%) to outweigh its possible side effects. By contrast, patients with RS $\geq$ 31 greatly benefit from chemotherapy due to their increased (28%) recurrence risk (54). The clinical management of intermediate risk patients is more varied and includes endocrine therapy with or without chemotherapy, depending on the patient's clinicopathologic characteristics and individual preference. Two ongoing clinical trials aim to further stratify the benefit of chemotherapy in patients with intermediate RS who are clinically node-negative (TailorX) or node-positive (RxSponder) at presentation.

Due to the costs, time and technical expertise required for molecular assays, IHC stains have been evaluated as possible alternative methods for indirect assessment of molecular subtype that can be used in most laboratories. The IHC staining panel comprising ER, PR, HER2, Ki-67, epidermal growth factor receptor (EGFR) and cytokeratin 5/6 (CK5/6) can identify the molecular subtypes of breast cancer with satisfactory and reproducible accuracy: (1) Luminal A (ER+/PR±/HER2-/Ki-67−); (2) Luminal B (ER+/PR±/HER2−/Ki-67+; with Ki-67-positivity defined as ≥14%); (3) Luminal/HER2+ (HER2+/ER+/PR ±); (4) HER2+ (HER2+/ER−/PR−); and (5) Basal, including core basal (ER−/PR−/HER2−/EGFR+ or CK5/6+), and five-marker negative (ER−/PR−/HER2−/EGFR−/CK5/6−) subgroups (55, 56). Considering that not all triple-negative tumors are basal-like and *vice versa*, and that ER-positive luminal tumors are highly diverse, genetic heterogeneity of breast cancer is likely far more complex than our current understanding of this multidimensional issue or the existing molecular classifications. Development of assays integrating multigene tests with mutational or genomic profiles is required to better elucidate the interplay and clinical significance of prognostic and predictive molecular drivers in ER-positive breast cancer (52).

INTRATUMOR HETEROGENEITY

Histopathologic Heterogeneity

Morphologic intratumor heterogeneity can be appreciated as variability in different areas of tumor (spatial heterogeneity), or as tumor progression over time (temporal heterogeneity) (1). Spatial heterogeneity is readily appreciated in daily surgical pathology practice within a single tumor, but can also be detected between primary breast carcinoma and synchronous lymph node metastases, and even between synchronous metastases from different sites. Breast carcinomas with truly mixed morphology consist of two morphologically different components (e.g., IDC and mucinous carcinoma), but other tumors exhibit ambiguous morphologic features (e.g., IDC with lobular features) or contain foci of distinct differentiation (e.g., IDC with focal squamous/basaloid or spindle cell differentiation) (**Figure 1**). Morphologically distinct areas within individual tumors can be clonal with specific genetic aberrations (57–59). Temporal heterogeneity includes evolution of an invasive tumor over time or in response to therapy (60, 61),

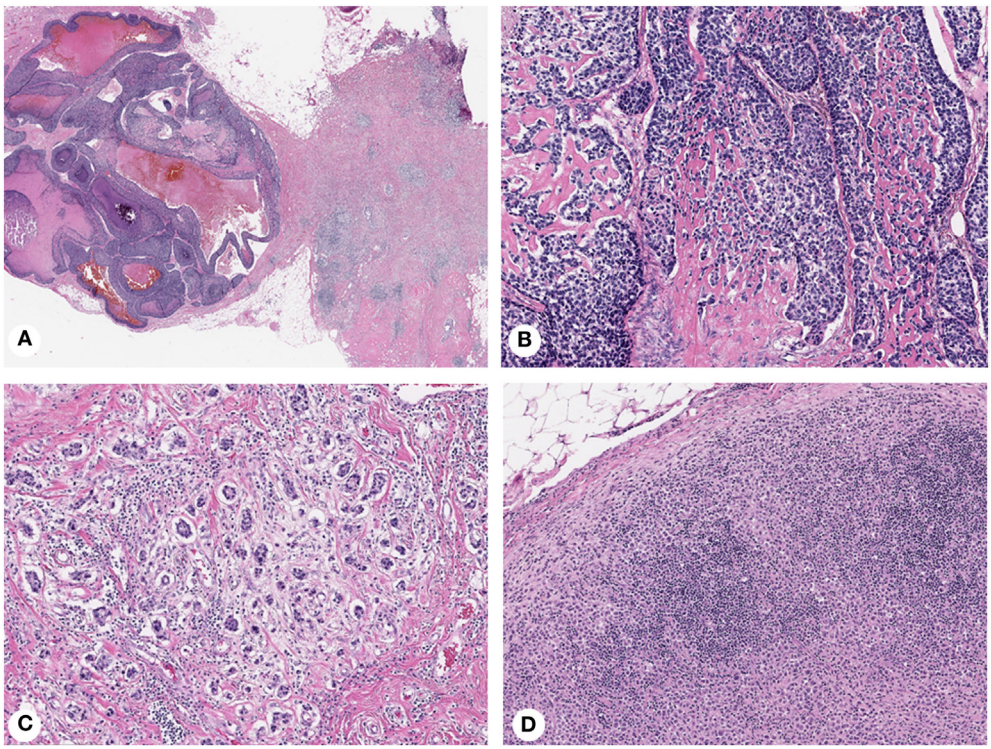

FIGURE 1 | Histopathologic heterogeneity of breast cancer: invasive mammary carcinoma with mixed morphology **(A)**, composed of basaloid areas with osteoid production **(B)** and ductal not otherwise specified **(C)** components. Lymph node metastasis showing a diffuse pattern of tumor growth **(D)**. Magnification: 100× **(A)**, 200× **(B–D)**; Hematoxylin-eosin staining.

development of asynchronous metastatic disease (62, 63) and progression from *in situ* to invasive carcinoma (64, 65).

Although current clinical management of breast cancer is guided by histologic, IHC, and molecular characteristics of the primary tumor, treatment efficacy may be affected by altered morphologic and IHC features in metastases (1, 66). Discordance rates include 16–33.6% for ER, 32–40% for PR, and 10–15.7% for HER2 (67–69). Furthermore, women with discordant ER-staining results between primary and metastatic breast carcinoma had a 48% increased risk of death in one study (69). Variability in biomarker expression between primary and metastatic tumors can be due to treatment (70) or may occur in the absence of therapeutic intervention (67, 69, 71, 72). Significant variations have also been reported in genomic heterogeneity (62, 73), single nucleotide or copy number variants (63, 66, 74, 75), and chromosomal rearrangements and insertion/deletions (75). Due to insufficient evidence that changing treatment based on the altered biomarker status affects patient outcome, the current practice guidelines only recommend biopsying and retesting ER/PR/HER2 on accessible metastases if clinically indicated (76).

Biomarker Heterogeneity

Expression of biomarkers can be highly variable within an individual tumor (**Figure 2**) causing interpretation problems and discordant results in small biopsies. ER/PR staining variations within a single tumor have long been recognized (77, 78). The proportion of ER/PR-expressing tumor cells in individual tumors varies from 1 to 100%, and expression levels directly correlate with response to endocrine therapy (26, 27). However, even tumors with very low levels (1% of tumor cells) may respond, justifying the use of the 1% cutoff for ER/PR-positivity by the ASCO/CAP guidelines (23). Nevertheless, this approach does not consider intratumor heterogeneity, accounting for limited clinical significance of classifying tumors with unequal distribution of ER-expressing cells as ER-positive (52).

Human epidermal growth factor receptor 2 (HER2) IHC staining and gene amplification can be highly heterogeneous (78–80) and affect disease-free survival (81). Discrepant HER2 IHC results ranges from 1 to >50% (79, 82, 83), while the rate of gene amplification heterogeneity is 5–30% (84). By IHC, HER2-positive tumors show complete, intense, circumferential membrane staining in 10–100% of tumor cells (3+ staining). Some tumors exhibit incomplete and/or weak-to-moderate circumferential membrane staining in >10% of cells or complete, intense, circumferential membrane staining in ≤10% of cells (2+ staining) by IHC but gene amplification by ISH (24). Some cases have protein overexpression without gene amplification, amplification without protein overexpression, or marked intratumor heterogeneity. Although the ASCO/CAP guidelines acknowledge heterogeneous amplification and recommend reporting separate areas (84), detecting gene amplification in one area is sufficient to consider a tumor HER2-amplified. This approach maximizes patient eligibility for targeted therapy without considering clinical implications of intratumor heterogeneity (52).

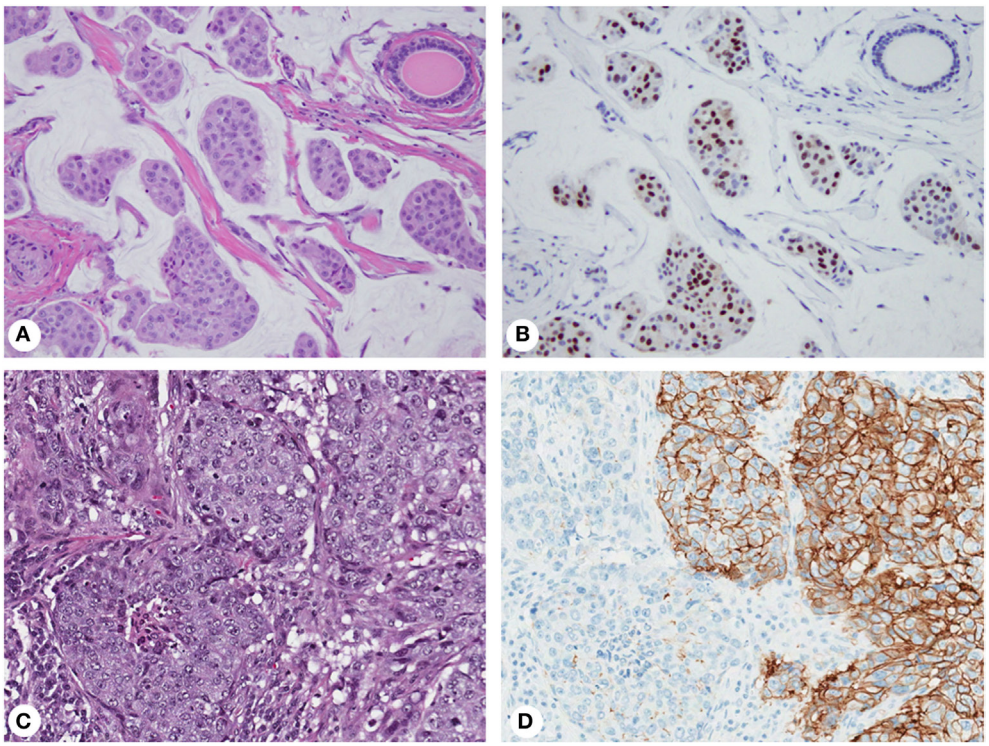

FIGURE 2 | Biomarker heterogeneity of breast cancer: mucinous carcinoma **(A)** with variable expression of estrogen receptor from no immunoreactivity to nuclear staining with weak to strong intensity **(B)**; invasive ductal carcinoma **(C)** with areas of 3+ (positive) and 1+ (negative) membranous staining for human epidermal growth factor receptor 2 **(D)**. Magnification: 200× **(A–D)**; hematoxylin-eosin staining **(A,C)** and immunohistochemistry **(B,D)**.

Other biomarkers with heterogeneous expression include EGFR (85), p53 (78, 85), c-myc (82), and proliferation markers, including Ki-67 (78, 85, 86), cyclin-D1 (82), and PCNA (87). Ki-67 is a non-histone nuclear protein expressed in all phases of the cell cycle except G0. It has been shown to have a prognostic and predictive value in both ER-positive and ER-negative breast carcinomas (88–90). However, expression levels of Ki-67 can be notoriously higher at the tumor periphery with variable staining throughout the tumor in the form of hot spots (91). Furthermore, intratumor heterogeneity of Ki-67 expression can occur in breast carcinomas of various histologic subtypes and grades (86). Several scoring systems have been suggested for the assessment of Ki-67 staining, including evaluating the hot spots alone, calculating the average score including hot spots, or even avoiding them altogether (91). In contrast to primary tumors, lymph node metastases have been reported to have a homogeneous distribution of Ki-67 expression. Moreover, metastatic tumor cells were highly proliferative and associated with Ki-67 levels in the highest expression hot spots in primary tumors. This may reflect the temporal heterogeneity through clonal expansion of the primary tumor growth fraction with metastatic potential (92).

It is unclear whether intratumor heterogeneity represents a true biologic phenomenon or a technical artifact due to poor fixation and/or processing (78, 93). Nonetheless, extensive sampling and IHC testing with adequate negative and positive controls are always prudent.

Circulating Tumor Cells (CTCs)

Circulating tumor cells are cancer cells that detach from a primary tumor and circulate in the bloodstream during cancer progression (94). CTCs have been reported in 26% of metastatic breast tumors (95). CTC count is an independent predictor of poor survival, treatment resistance, and early recurrence in some studies (96–104). However, practical application of CTC-based assays as "liquid biopsies" is limited by significant molecular and functional heterogeneity of CTCs (105–107), including variability at the protein (HER2, ER, Ki-67) (76, 108–114) and gene (PIK3CA) levels (76, 108–114), and EMT (115, 116). During the process of EMT, which is thought to precede the development of lymphovascular invasion and metastasis, the tumor cells lose epithelial characteristics, such as cell polarity, cell-to-cell adhesion, and expression of epithelial markers (EpCAM), and acquire mesenchymal properties including motility and invasiveness (115, 116). The presence of EMT in CTCs indicates a poor prognosis (117). Discordant HER2-expression in CTCs in particularly relevant (118–121), and clinical trials (DETECT, TREAT-CTC) are underway to evaluate treatment options based on the HER2 status of CTCs in metastatic breast cancer (122, 123). Heterogeneity in CTCs is thought to represent one of the mechanisms of resistance to endocrine therapy (1). Nonetheless, due to insufficient clinical evidence, the ASCO guidelines do not recommend changing therapy solely on the basis of CTC counts for monitoring treatment response (76).

Genetic Heterogeneity

Breast cancer shows considerable intratumor heterogeneity with regard to chromosomal and genomic alterations (44, 124–130) which affect many processes and functions, such as signaling pathways, antitumor immunity, cell senescence, migration and metastasis, angiogenesis, treatment response, and metabolic pathways (52). Different cell clones can either segregate in different areas of the tumor or scatter and intermingle within the same area (131). Complexity of intratumor genetic heterogeneity is best exemplified by a study of 100 tumors which identified driver mutations in >40 cancer genes, including AKT2, ARID1B, CASP8, CDKN1B, MAP3K1, MAP3K13, NCOR1, SMARCD1 and TBX3, and 73 combinations of mutated genes (129). Intratumor genetic heterogeneity can be characterized by bulk sequencing and single-cell or single-molecule sequencing (132). Bulk tumor sequencing cannot determine the cellular origin of molecular changes, location within tumors or the degree of heterogeneity, while single-cell sequencing cannot provide information on the remaining cell population, limiting their clinical use in clinical practice (52). An autopsy study comparing the molecular alterations in multiple synchronous metastases of breast carcinoma documented molecular evolution and clone selection of tumor cells in response to targeted treatment, and highlighted the challenges to targeted treatment posed by the complex molecular heterogeneity of metastatic disease (133).

Non-Genetic (Epigenetic) Heterogeneity

Epigenetic heterogeneity is defined as modifications in gene expression without DNA sequence changes (52, 134, 135). In breast cancer, epigenetic silencing through histone modification or DNA methylation can affect tumor suppressor genes including p16INK4A (136) and RASSF1A (137), and ER/PR/HER2 (138). Transient phenotypic variants of cells can also arise due to stochastic changes in the biochemical processes within cells (135), which might involve changes in chromatin states or mRNAs (139) and affect sensitivity to therapy (139). The clinical significance of non-genetic heterogeneity remains to be determined.

FOUR MECHANISMS OF BREAST CANCER HETEROGENEITY

Differentiation State of the Cell-Of-Origin

Each mammary cell type has a specific molecular profile (140, 141). Tumor phenotype is determined by the combination of this differentiation state and the tumor-initiating genetic event. Distinct differentiation states of human mammary epithelial cells grown in cell cultures lead to different tumor subtypes in mouse xenografts (142, 143), e.g., EpCAM+ cells form epithelial tumors with variable ER-positivity, while CD10+ cells are precursors of metaplastic carcinoma (144). Multiple phenotypes can arise from one cell-of-origin depending on the initiating genetic event, e.g., HER2-expression in luminal cells forms luminal tumors, while BRCA1/2 leads to basal differentiation (145, 146). Furthermore, expression of the same oncogene (e.g., PIK3CA) in luminal cells can lead to different tumor types (147), while BRCA2/TP53 depletion results in IDC and metaplastic carcinoma in luminal cells, but myoepithelial carcinoma in basal cells (141). Nevertheless, the final tumor phenotype does not always reflect the cell-of-origin (141).

Cell Plasticity

The equilibrium of cell states within tumors is maintained by dynamic bidirectional cell conversions between "cancer stem cells" (CSCs) and non-CSCs (148). CSCs self-renew and form more stem cells, differentiated cells, and tumor cells (149), while differentiated tumor cells can dedifferentiate (150). Cell plasticity may involve EMT and PIK3CA-expression (147, 151, 152).

Genetic Evolution of Cancer

Tumorigenesis is a multi-step evolutionary process driven by Darwinian selection of the fittest cells and genetic instability (149, 153). Although most tumors arise from a single cell due to the initiating genetic event ("driver mutation"), cancer cells acquire additional aberrations during tumor evolution and, thus, each tumor contains multiple subclones harboring "passenger mutations" (132). Cell plasticity and genetic evolution may overlap as CSCs evolve and change in frequency due to clonal evolution during tumor progression (149).

Tumor Microenvironment

Tumor stroma contains fibroblasts, blood vessels, and immunocompetent cells. Interactions between this non-cancerous microenvironment and tumor cells can contribute to carcinogenesis (154), exemplified by decreased sensitivity of tumor cells to growth inhibitors (155) and suppressed tumor growth by microvasculature (156).

Clinical Implications

Despite our improved understanding of complex phenotypic and genetic aspects of tumor heterogeneity, no significant clinical progress has been made with regards to incorporating this knowledge into effective diagnostic, prognostic, and therapeutic strategies for breast cancer (52). Patients are managed based on the ER/PR/HER2 status of the primary tumor, and metastatic sites may not always biopsied for histologic confirmation or biomarker retesting (68). Since "actionable" mutations in the initial tumor may no longer be responsible for tumor progression, it is essential to identify the dominant clones driving metastatic disease and treatment resistance (157, 158). Ideally, intratumor heterogeneity should be assessed by sequencing technologies at diagnosis for each patient, followed by monitoring of clonal dynamics during disease progression and treatment. This will allow for the identification of genetic changes driving resistance as well as therapy adjustments (1, 141, 159). Potential strategies to overcome treatment resistance include targeting driver mutations and deleterious passenger mutations, and modulating the tumor microenvironment and immunotherapy (93). Further well-designed studies are required to elucidate the clinical validity of rapidly accumulating data.

AUTHOR CONTRIBUTIONS

GT: writing original draft and editing. EB: writing, reviewing, and editing.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.