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Epithelial–Mesenchymal Transition Programs and Cancer Stem Cell Phenotypes: Mediators of Breast Cancer Therapy Resistance

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ABSTRACT

Epithelial–mesenchymal transition (EMT) programs play essential functions in normal morphogenesis and organogenesis, including that occurring during mammary gland development and glandular regeneration. Historically, EMT programs were believed to reflect a loss of epithelial gene expression signatures and morphologies that give way to those associated with mesenchymal cells and their enhanced migratory and invasive behaviors. However, accumulating evidence now paints EMT programs as representing a spectrum of phenotypic behaviors that also serve to enhance cell survival, immune tolerance, and perhaps even metastatic dormancy. Equally important, the activation of EMT programs in transformed mammary epithelial cells not only enhances their acquisition of invasive and metastatic behaviors, but also expands their generation of chemoresistant breast cancer stem cells (BCSC). Importantly, the net effect of these events results in the appearance of recurrent metastatic lesions that remain refractory to the armamentarium of chemotherapies and targeted therapeutic agents deployed against advanced stage breast cancers. Here we review the molecular and cellular mechanisms that contribute to the pathophysiology of EMT programs in human breast cancers and how these events impact their "stemness" and acquisition of chemoresistant phenotypes.

Introduction

With over 260,000 newly diagnosed cases in 2017, breast cancer remains the most commonly diagnosed malignancy among women in the United States (1). Although recent advances in treatment have produced a moderate decline in the mortality rate associated with breast cancer (2), this disease nevertheless claims the lives of over 42,000 women each year, making it the second most common cause of cancer-related death among women (1). The acquisition of metastatic and recurrent phenotypes is responsible for the death of approximately 90% of patients with breast cancer (3–5). Indeed, metastases are typically incurable and result in a median survival of only 1.5 to 3 years for patients with breast cancer. Unfortunately, our understanding of how breast cancers become metastatic remains poor, as does our knowledge of how disseminated breast cancer cells escape clinical detection by remaining dormant for years before reemerging as chemoresistant and incurable secondary tumors (6, 7). The challenge associated with detecting disseminated malignancies is highlighted by the fact that autopsies clearly show that the majority of trauma patients harbor undiagnosed and asymptomatic occult neoplastic and micrometastatic lesions (8–10). Along these lines, circulating tumor cells were readily detected in 36% of patients with breast cancer that appeared to be disease-free and within stable remission for 7–22 years after undergoing mastectomy (11). Similarly, nearly 25% of breast cancer survivors still housed circulating genomic DNA matching that of their primary tumors up to 12 years following disease diagnosis (12). These findings, together with the fact that approximately 62% of breast cancer–related deaths occur 5 to 20 years after initial diagnosis (8) imply that latent breast micrometastases play a pivotal role in the majority of breast cancer–associated mortalities. Although recent genetic and epigenetic analyses have begun to reveal the molecular landscapes in metastatic breast cancers, these efforts have yet to be tailored specifically to dormant disseminated breast cancer cells and their reactivation within the metastatic niche during disease recurrence. It should be noted that the acquisition of metastatic and chemoresistant phenotypes typically proceeds in a coincident manner. Indeed, metastatic tumors are generally refractory to chemotherapies that were originally effective in primary tumor settings, while poor response rates in neoadjuvant settings portend more rapid rates of metastatic recurrence and shortened overall survival (13).

Many of the aforementioned processes are exemplified in cells that have undergone an epithelial–mesenchymal transition (EMT) program, a cellular state that encompasses several plasticity-associated phenotypes, including stemness, metastatic dormancy, and chemoresistance. Here we review the risk factors that influence breast cancer development and the heterogeneous nature of this disease. In addition, we also discuss recent findings that impact our understanding of the interplay between EMT programs, breast cancer stem cells (BCSC), and chemoresistant phenotypes, as well as how these events impact disease recurrence and the clinical course of patients with breast cancer.

Breast Cancer Risk Factors

The epidemiological risk factors influencing the development of breast cancer are complex and multifold. Historically, the probability of developing breast cancer has been thought to be largely dependent upon the activity of the steroid hormone estrogen (14). The mechanistic foundations for the oncogenic activities of estrogen include its mitogenic and anti-apoptotic properties in mammary epithelial cells (MECs; ref. 15), as well as the carcinogenic properties of its quinone metabolites (16). As such, factors that augment cumulative lifetime exposure to estrogen (e.g., early age at menarche, late age at

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menopause, absence of breastfeeding, etc.) increase the risk of breast cancer (17). Nonetheless, generalizing the physiologic response of MECs to estrogen appears to be a gross oversimplification, as multiple studies reveal that physiological variation of breast composition (i.e., varying proportions of luminal, myoepithelial, and stromal cells) can alter the oncogenic effects of estrogen (18–20). Indeed, these findings could explain recent epidemiologic studies that contradict the breast cancer–promoting effects of early menarche and absence of lactation on the development of hormone-dependent subtypes of breast cancer (17, 21). Moreover, although less understood mechanistically, the risk of developing breast cancer is thought to be influenced by several lifestyle and modifiable factors, including direct correlations with body mass index (22) and alcohol consumption (17), and indirect correlations with physical activity (23).

Finally, a subset of breast cancers (5–10%; ref. 24) are linked directly to heritable germline mutations in the tumor suppressors BRCA1 and BRCA2, which function in maintaining genome integrity by responding to and overseeing the repair of damaged DNA (25). Although constituting a small fraction of breast cancers, BRCA1/2 mutations nonetheless represent the largest known predictor for the development of breast cancer, with carriers that harbor defects in these DNA repair genes exhibiting approximately 70% cumulative risk of developing breast cancer by age 80 (26). Thus, continuing to enhance our understanding of the complex interplay between hereditary and environmental risk factors for breast cancer could aid in lowering the prevalence of this disease, which carries a lifetime risk exceeding 12% in women.

Breast Cancer Heterogeneity

The clinical management of breast cancer is complicated by its manifestation as an exceptionally heterogeneous disease. In fact, the extensive degree of molecular and histopathologic variation demonstrated by breast cancers precludes their classification as a single and uniform disease. Instead, the proliferation of genomic analyses has established that breast cancers are composed of a diverse collection of diseases that possess varying clinical prognoses and require distinct treatment strategies. Along these lines, the cells comprising an individual breast tumor frequently exhibit substantial molecular and phenotypic dissimilarities, resulting in intratumor heterogeneity that further complicates prognosis and the response to therapies.

Clinical targeting of hormone receptors

The physiologic architecture of the mammary gland, including the growth and development of its ductal system and lobule units, is highly dependent upon the ovarian steroid hormones estrogen and progesterone (27). Luminal MECs respond to these hormones via their cognate receptors, namely estrogen receptor α (ERα) and progesterone receptor (PR). Despite their clear physiologic role in mammary gland homeostasis, it has long been understood that the functions of ERα and PR are frequently hijacked by malignant cells during mammary tumorigenesis (28).

Historically, the oncogenic activities of estrogen are believed to drive the growth and progression of hormone receptor–dependent breast cancers. Indeed, the detrimental role of estrogen can be traced to the earliest steps of mammary tumorigenesis, including the initial carcinogenic insults imposed on mammary tissue (29, 30). More importantly, estrogen also elicits oncogenic activity by controlling several MEC signaling networks. For instance, mutations in ERα, as well as aberrant expression of its coactivators, can circumvent the ligand-dependent canonical functions of ERα and lead to dysregulated ERα-dependent signaling wherein the expression of various mitogenic and antiapoptotic genes (e.g., MYC, cyclin D1, BCL2) are constitutively activated (31, 32). In addition, ERα can elicit oncogenic signals through extranuclear methods, doing so by associating with the plasma membrane where it modulates the mitogenic activities stimulated by the EGFR, and by the PI3K/AKT signaling axes (33).

Because of the established efficacy of ERα-targeted therapies in the treatment of breast cancer, the vast majority of studies have focused their attention on the role of estrogen and ERα in driving mammary tumorigenesis. However, recent evidence strongly implicates PR in exerting a substantial impact on breast cancer development, an unsurprising finding given that estrogen-dependent proliferation of the pubertal mammary gland requires intact progesterone signaling and progesterone is responsible for the proliferation and expansion of mammary tissue during the luteal phase of the menstrual cycle (34). Accordingly, oncogenic activities of PR are coupled to its activation of cyclin D1 transcriptional complexes and subsequent induction of cell-cycle progression (35) as well as the PR-dependent secretion and stabilization of RANKL (TNFSF11), a cytokine that acts in a paracrine fashion to initiate cell-cycle progression in luminal MECs (36).

Regardless of the precise mechanisms by which breast cancers depend upon hormone receptors, the therapeutic modification of these receptors, particularly ERα, represents one of the most successful efforts in targeted cancer therapy to date. Currently, standard-of-care therapy necessitates IHC evaluation of ERα and PR status in breast lesions, a measure that dictates the eventual utility of endocrine therapy. Inhibiting the activity of ERα is accomplished through targeting strategies, including direct approaches using selective ER modulators (SERMs; e.g., tamoxifen) and selective ER degraders (SERDs; e.g., fulvestrant; ref. 37). Alternatively, the oncogenic activities of ERα can also be inhibited indirectly via suppression of estrogen synthesis using aromatase inhibitors, an approach that possesses particular clinical utility in postmenopausal women (38). Similarly, estrogen production can be restricted through the use of luteinizing hormone–releasing hormone (LHRH or GNRH1) agonists to indirectly repress ovarian function and their synthesis of estrogen in premenopausal women (38). Although treatment strategies depend heavily on tumor staging characteristics, implementation of adjuvant hormonal therapy has successfully lowered the recurrence and mortality rate in patients harboring hormone receptor–positive breast carcinomas (39).

Clinical targeting of HER2

The heterogeneity of breast cancers is further exemplified by the frequent clinical utilization and characterization of the receptor tyrosine kinase (RTK) HER2/ErbB-2, which belongs to the EGFR family of membrane-associated RTKs (e.g., EGFR, HER2, HER3, and HER4). Intracellular signaling propagated by HER2 requires its heterodimerization with other EGFR members, particularly HER3 (40), leading to the phosphorylation of HER2 within its protein kinase domain and subsequent activation of the PI3K/AKT, MAPKs, and PLCγ (PLCG1)/PKC (PRKC) signaling axes that coalesce in promoting the proliferation, metabolism, and survival of MECs (41). The oncogenic activities of HER2 therefore transpire secondary to the overexpression of itself or its heterodimer partners and through the acquisition of mutations that promote constitutive activity of its downstream signaling pathways (42). All breast carcinomas are currently subjected to IHC- and/or *in situ* hybridization (ISH)-based quantification of HER2 expression because (i) HER2 amplification occurs in approximately 20%–25% of breast cancers and portends the presence of aggressive disease phenotypes coupled to significantly diminished disease-free

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and overall survival (43), and (ii) the expression status of HER2 represents an important predictive factor that dictates its targeting and inactivation via either of two pharmacologic strategies (41), namely the administration of small-molecule tyrosine kinase inhibitors (TKI) or targeted mAbs.

As a class of pharmacologic agents, TKIs are compounds that interact competitively with the ATP-binding domain of RTKs, thereby impeding their phosphorylation and activation of several signaling cascades. The most widely explored TKI in the treatment of $HER2^+$ breast lesions has been lapatinib, a dual-specific TKI that inhibits the activation of both HER2 and EGFR, and in doing so, abrogates signaling through major oncogenic pathways, such as MAPKs, PI3K/AKT, NFκB, and IGF1 (44). Additional TKIs that have shown some efficacy in treating $HER2^+$ breast cancers include the EGFR-specific inhibitor, gefitinib (45, 46), as well as the HER2-specific inhibitor, tucatinib, that has recently been granted FDA approval to be combined with trastuzumab and capecitabine regimens for the treatment of patients with progressive, metastatic $HER2^+$ breast cancer (47).

Although TKIs possess a degree of clinical efficacy, the therapeutic pillar for targeting $HER2^+$ breast cancers has been mAbs directed against HER2. The most well-characterized anti-HER2 mAb is trastuzumab, which is a recombinant humanized mAb directed against the extracellular Domain IV of HER2 (48), thereby (i) preventing the formation of ligand-independent HER2 homo- and heterodimers (49); (ii) promoting the internalization and degradation of HER2 (50); and (iii) stimulating $CD8^+$ T-cell cytolytic activity or NK-cell–mediated antibody-dependent cellular cytotoxicity toward $HER2^+$ mammary cancer cells (51). Collectively, these mechanisms coalesce in mediating the substantial clinical efficacy of trastuzumab against $HER2^+$ breast cancers, including superior outcomes compared with lapatinib when used in combination therapies against advanced disease stages (52).

More recently, an analogous HER2-directed recombinant humanized mAb, pertuzumab, has gained traction in clinical settings. Pertuzumab binds to extracellular Domain II of HER2, an epitope that is farther from the plasma membrane and does not overlap with the Domain IV epitope recognized by trastuzumab (53). Importantly, the distinct receptor sequences targeted by pertuzumab and trastuzumab enables their combined administration in clinical settings, a strategy that significantly extends the overall survival in patients with $HER2^+$ breast cancer with advanced disease as compared with those solely receiving trastuzumab (54). Thus, despite the aggressive disease phenotype associated with HER2 amplification, the employment of HER2-targeted therapies has in many respects equalized the survival outcomes of luminal B patients (i.e., ER^+ and $HER2^+$) with their luminal A counterparts (43).

Clinical efforts to target TNBCs and their heterogeneity

The multiple permutations that define ER/PR/HER2 receptor status of breast cancers exemplify the heterogeneity of this disease, thus necessitating the development of subtype-specific treatments tailored to individual patients. In fact, the presence of these receptors continues to represent a highly advantageous trait, serving as some of the most consistently predictive and actionable molecular targets in all of oncology (55). As such, the largest clinical burden associated with breast cancer stems from a subset of patients harboring lesions known as triple-negative breast cancer (TNBC), which lack expression of ERα, PR, and HER2 and constitute approximately 15% to 20% of all diagnosed breast cancers (56). TNBCs are consistently found to exhibit higher disease grades and elevated proliferative indices as compared with other non-TNBC subtypes (57, 58) and consistently harbor a high and irregular mutational burden that results in aberrant signaling through a wide variety of pathways (54). As such, patients with TNBC endure the worst progression-free and overall survival rates of all breast cancer subtypes; they are also particularly prone to disease relapse and early death within 5 years of initial diagnosis and treatment (59).

Although targeted therapies against TNBCs have yet to be developed, this breast cancer subtype is significantly more sensitive to chemotherapy regimens as compared with their non-TNBC counterparts (60). Currently, taxane- and anthracycline-based therapies are effective and remain the most commonly used agents for TNBC treatment (61); however, several unique strategies are being evaluated in clinical settings, most notably (i) the expansion of platinum-based regimens (i.e., carboplatin and cisplatin); (ii) the implementation of PARP inhibitors, particularly in TNBCs defective in DNA repair mechanisms (e.g., BRCA1/2 mutations); (iii) novel combination therapies that include histone deacetylase inhibitors; and (iv) more than 50 ongoing clinical trials exploring the utility of immune checkpoint inhibitors in various combinations and clinical settings (61, 62).

The vast diversity of mechanisms of action and pathways targeted by the agents encompassed in current TNBC treatment paradigms epitomizes the heterogeneity of this aggressive subtype. Accordingly, gene expression analyses of 386 TNBC tumors enabled the categorization of six genetically distinct subtypes that display unique biological characteristics, namely basal-like 1 (BL-1) and 2 (BL-2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR; ref. 63). The molecular classification of these TNBC subtypes was initially met with enthusiasm, as they provided the first opportunities to stratify TNBC patients into prognostic or predictive groups. Indeed, a retrospective study of human TNBCs demonstrated that pathologic complete response to neoadjuvant taxane- and anthracycline-based therapy was highly dependent upon TNBC subtype (64). However, more recent attempts to extend these signatures to additional clinical settings have proven to be disappointing in their ability to effectively stratify patients based on overall prognosis and likelihood to respond to predicted subtype-specific chemotherapy regimens (65). Ongoing efforts by several groups to consolidate and refine these TNBC subtypes has shown reasonable success in better informing the design of rational therapeutic strategies for this highly vulnerable disease population (65, 66).

Intrinsic molecular subtypes and their clinical implications

Historically, the IHC determination of ERα, PR and HER2 expression status, as well as *in situ* hybridization for HER2 amplification in breast carcinomas have provided limited insights into the heterogeneous nature inherent in mammary tumors. With the advent of gene expression profiling techniques (e.g., microarray and RNA-seq technologies), breast cancers have been further classified into distinct groups based on their transcriptional signatures. Indeed, Perou and colleagues (67) utilized DNA microarrays to analyze the transcriptomes of primary human breast cancers, resulting in the identification of five intrinsic breast cancer clusters comprised of luminal A, luminal B, HER2-enriched, basal, and normal breast-like (67). Importantly, molecular breast cancer subtypes are tightly associated with the histologic subtypes discussed above, making them a valuable resource to further define the molecular heterogeneity of breast cancers. For instance, while luminal A and luminal B tumors are tightly linked to tumors that express ERα and PR, both luminal designations typically exhibit a gradient of ERα expression with luminal B tumors generally harboring lower quantities of ERα as compared with their luminal A counterparts (68, 69).

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Such precise molecular characterization of breast cancer tissue affords a unique degree of clinical utility to intrinsic subtyping. Currently, practical determination of the intrinsic subtype of breast carcinomas is accomplished using a minimized set of genes (i.e., 50 genes) in a practice called PAM50 profiling (70). Numerous studies have attributed substantial prognostic and predictive value to deciphering PAM50 signatures (69, 71–74). For example, PAM50 profiling has proven to be extremely beneficial in differentiating luminal A from luminal B subtypes, as these subtypes are typically indistinguishable using traditional IHC-based methods (i.e., both are ER^+/PR^+) despite the fact that luminal B lesions are consistently associated with worse disease-free survival outcomes and are frequently more chemosensitive (75). PAM50 signatures can, therefore, stratify ER^+/PR^+ patients based on their likelihood of disease recurrence or their potential response to neoadjuvant chemotherapy (75), a feature lacking during the simple assessment of ERα and PR status. Finally, PAM50 has been approved for assessing the 10-year risk of distant recurrence in stage I or II breast cancer that express ERα and PR (69).

EMT Programs

In the most general sense, oncogenic EMT programs reflect an organized transdifferentiation process whereby polarized epithelial cells shed their immotile behaviors to acquire fibroblastoid-like phenotypes characterized by increased invasive and migratory capabilities that compel indolent carcinoma *in situ* lesions to become highly aggressive invasive lesions (3, 76, 77). The induction of EMT is largely dependent upon factors secreted and entrapped in the tumor-associated stroma, most notably TGFβ, platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), and EGF (77, 78). These paracrine factors elicit the activation of a host of EMT-inducing transcription factors [e.g., TWIST1, SNAIL (SNAI1), SLUG (SNAI2), ZEB1) that collectively act to promote the acquisition of EMT phenotypes by suppressing the expression of epithelial-associated molecules [e.g., E-cadherin (CDH1) and zona occludens 1 (TJP1)] and inducing those associated with mesenchymal traits [e.g., N-cadherin (CDH2), fibronectin, and vimentin; ref. 79]. Indeed, many mediators of EMT exhibit clinical relevance in patients with breast cancer, including findings that robust expression of (i) SNAIL correlates with tumor recurrence; (ii) SLUG associates with metastatic progression and disease recurrence; and (iii) SLUG and TWIST1 both portend to poor tumor grade and diminished overall survival (80, 81).

The invasive and migratory features conferred to primary breast cancer cells via EMT programs remain a critical juncture during the systemic dissemination of these cells (3, 77). Moreover, EMT programs encompass a conglomeration of numerous cell states besides those underlying enhanced migratory and invasive properties. Indeed, upon traversing an EMT program, breast carcinoma cells are endowed with enhanced survival, resistance to chemotherapy, and immune tolerance; they also acquire dormancy- and stemness-associated phenotypes (see below; refs. 3, 77). Despite intensive investigation into the molecular underpinnings of EMT programs, the mechanisms related to the precise timing, location, and cell populations susceptible to engage in EMT programs remains to be fully clarified. For instance, the initial invasion and migration of primary tumor cells often unfolds through the collective migration of sizeable cell clusters that penetrate into the surrounding parenchyma as opposed to the liberation of individual cells (82). Although seemingly at odds with a model of single-cell dissemination as predicted by EMT programs (5), it is important to note that (i) EMT is a dynamic and plastic process that enables cells to exist along a spectrum of EMT phenotypes (i.e., incorporation of migratory features, while maintaining adhesion complexes), and (ii) carcinoma cells located at the leading migratory edge of collectively migrating tumors do in fact exhibit post-EMT and mesenchymal-like phenotypes that not only function to degrade the obstructing stroma, but also to compel the trailing tumor mass to migrate in a collective manner (5, 83). The role of EMT programs to promote the self-renewal, expansion, and chemoresistance of breast cancer stem cells is discussed in the succeeding sections.

Breast cancer stem cells

Emerging evidence indicates that the successful metastatic colonization of distant tissues by breast cancers may require the presence of a unique class of malignant cells that are highly specialized and proficient at initiating tumor outgrowth, namely breast cancer stem cells (BCSC). By definition, BCSCs must possess the capacity to (i) preferentially initiate tumorigenesis, (ii) undergo self-renewal, and (iii) differentiate into the various heterogeneous subsets of cells that recapitulate those found in the bulk population of the primary tumor (84). Indeed, multiple animal models have established that the metastases produced by breast cancers depend upon BCSCs that function in concert with a tumor microenvironment that is conducive to the acquisition of BCSC phenotypes (85–87). Moreover, occult breast cancer metastases disproportionately harbor gene expression profiles that are enriched for pathways that define BCSCs (88), implying that the early colonization of foreign and stress-inducing microenvironments depends upon BCSCs. As such, the presence of BCSCs is required within distant tissues to act as founders for the colonization and subsequent metastatic outgrowth of disseminated breast cancer cells (89).

Interestingly, there exists significant overlap in the properties that define BCSCs as compared with those inherent in dormant disseminated carcinoma cells. Generally, both BCSCs and dormant cells are (i) notably proficient in surviving the stresses associated with foreign microenvironments and chemotherapeutic insult (90, 91); (ii) prone to residing for long periods of time in a state of quiescence (92); (iii) uniquely capable of evading immune detection (93); and (iv) highly responsive to signals derived from the metastasis-associated vasculature (93, 94). More specifically, dormant breast cancer cells isolated from random bone marrow aspirates obtained from patients with early-stage breast cancer were found to harbor traits indicative of BCSCs (95). Moreover, preclinical animal models have shown that dormant disseminated breast cancer cells consistently upregulate and activate pathways and molecular mediators critical to the maintenance of stemness (88, 96). Collectively, these findings imply that metastatic colonization relies heavily upon the phenotypic plasticity of disseminated tumor cells, particularly their ability to acquire dormancy- and CSC-like states.

Therapeutic Resistance in Breast Cancer

The initial pharmacologic treatment of breast cancer is dictated primarily by the receptor status and stage of the primary lesion at the time of disease presentation (i.e., size of primary tumor, involvement of regional lymph nodes, and presence of distant metastases). However, the development of therapeutic resistance to first-line treatment regimens represents a critical and significant barrier that negatively impacts the prolonged efficacy of standard-of-care treatment modalities, and consequently, overall patient survival. The following sections discuss the underlying mechanisms that confer breast cancer cells resistance toward the most frequently administered therapies against

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breast cancers, focusing specifically on the role of cellular plasticity (e.g., dormancy, EMT, and CSC phenotypes) in mediating these untoward events.

General mechanisms of resistance

Compounding the challenges that physicians face, the mechanisms responsible for targeted and chemotherapeutic resistance are immensely diverse, but generally fall into two distinct categories: (i) *de novo* resistance, or (ii) acquired resistance. *De novo* resistance is synonymous with intrinsic resistance and reflects the activation of inherent preexisting cellular defense mechanisms present at onset of first-line therapy. In stark contrast, acquired resistance reflects the genetic and epigenetic alterations that arise in carcinoma cells in response to specific pharmacologic agents or treatment regimens (97). The actions of these resistance mechanisms and their connections to cellular plasticity are discussed below.

Acquired resistance

Although often transpiring in an overlapping manner, *de novo*, and acquired resistance mechanisms can be differentiated from one another based on the unique fundamental traits associated with either form of resistance. Importantly, due to the relative ease of establishing therapeutically resistant carcinoma cell lines following their chronic *in vitro* treatment with anticancer agents, the mechanisms operant in acquired resistance are more clearly characterized than those in *de novo* resistance (98). For instance, administration of anticancer agents to carcinoma cells readily upregulates their expression of drug efflux pumps, particularly members of the ATP-binding cassette family (e.g., P-glycoprotein (ABCB5)). In fact, the upregulation and activation of drug efflux pumps underlies the ineffectiveness of nearly 33% of all anticancer drugs, including anthracyclines, vinca alkaloids, and taxanes (99). Additional means undertaken by breast carcinoma cells to circumvent the cytotoxic activities of chemotherapies include their ability to (i) upregulate the expression of antiapoptotic proteins; (ii) sequester drugs within intracellular compartments, thereby preventing access to their molecular targets; (iii) induce the expression of enzymes that metabolize and detoxify the drug; (iv) enhance the activation of DNA repair mechanisms in response to genotoxins; and (v) acquire mutations in molecular targets that render anticancer agents ineffective (100). Thus, while the mechanisms of acquired resistance are quite diverse, the initiation of these events generally relies on the intrinsic malleability of cancer cells to readily adapt to cytotoxic stressors and remain viable (101). Indeed, this plasticity reflects alterations in the epigenetic and genomic landscapes of breast carcinoma cells, which derive from defects in cell-cycle checkpoints, DNA repair mechanisms, and increased proliferation rates that coalesce to create a state of genomic instability.

De novo resistance

De novo mechanisms of resistance are often difficult to differentiate from those coupled to acquired resistance. For example, intratumoral heterogeneity can mask *de novo* resistance when small subpopulations of cells intrinsically harbor resistance-promoting mutations prior to first-line therapy. Indeed, such clonal variants manifest as residual disease that can become clinically relevant months-to-years after the initial therapy, thereby mimicking the temporal aspects of acquired resistance (102). However, certain mechanisms are clearly disparate and more commonly associated with *de novo* resistance. For instance, P-glycoprotein is expressed at high levels in numerous normal tissues, especially the colon, liver, kidney, and breast. As such, the tissue-specific distribution of drug efflux transporters plays a pivotal role in the *de novo* resistance in carcinomas derived from these tissues, as these channels are inherently poised to protect tumors from pharmacologic insults (103). More importantly, interactions between the tumor microenvironment and the developing carcinoma allow for unique *de novo* mechanisms of therapeutic resistance that are driven primarily through integrin-based cell adhesion networks that activate cell survival programs coupled to chemoresistance (e.g., cell adhesion–mediated drug resistance; refs. 104, 105). Thus, the stromal composition of metastatic microenvironments can provide disseminated tumor cells a survival sanctuary to resist clinical regimens.

Dormancy-induced chemoresistance

Dormant cells have long been recognized as inherently resistant to commonly used chemotherapeutic drugs (106). The chemoresistant traits of dormant cells stem from their natural propensity to assume quiescent phenotypes, an event that effectively abolishes the utility of chemotherapeutics designed to target highly proliferative and metabolically active carcinoma cells (107). Moreover, anticancer agents can select for a subset of dormant cells that are enriched in the expression of genes operant in regulating cell survival and multidrug resistance, implying that cytotoxic insults can select for a population of cells that are poised to instigate posttherapy relapse (93, 108). Along these lines, dormant cells can also utilize a distinct set of prosurvival tactics that are uncoupled from their quiescent states. For instance, disseminated dormant cells rely heavily upon the activation of the unfolded protein response as a means to reduce the expression of proapoptotic molecules, while simultaneously increasing that of pro-survival molecules, such as BiP (109). Indeed, this event may transcend involvement of the ER stress response to be elicited upon exposure to an array of cytotoxic therapies (110). Finally, dormant cells can also coopt many of pro-survival and chemoresistant features exploited by BCSCs (ref. 94; see below), once again underscoring the close link between dormant cells and CSCs described above.

EMT and stemness in breast cancer chemoresistance

The general molecular pathways exploited by breast cancer cells undergoing EMT and those residing in a stem-like state have been shown to be highly overlapping. Indeed, the seminal findings by Mani and colleagues (167) and Morel and colleagues (111) demonstrated that driving immortalized HMECs (human mammary epithelial cells) to traverse the EMT program results in the emergence of a population of mesenchymal cells that possess the molecular and phenotypic behaviors reminiscent of BCSCs. Moreover, EMT and stem-like phenotypes are also linked in a physiologic manner, as the EMT-mediator SLUG is enriched in normal mammary stem cells and, in conjunction with SOX9, is capable of converting differentiated MECs into mammary stem cells (112). More importantly, the mechanistic parallels between BCSCs and EMT programs frequently coalesce on pathways coupled to the acquisition of therapeutic resistance in breast cancer cells. Indeed, transcriptional profiling of endocrine- or chemotherapy-resistant human breast cancers identified gene expression patterns consistent with those detected in BCSCs. Importantly, these residual (i.e., resistant) breast cancer lesions were predominantly of the claudin-low subtype, a subset of breast cancer that is largely mesenchymal in nature (113). Collectively, these findings imply that common treatment strategies will be ineffective in breast cancers that have undergone an EMT program and reside in a chemoresistant stem-like state. In the succeeding sections, we highlight the impact of EMT programs and BCSCs to elicit resistance toward some of the most commonly used drugs in the treatment of breast cancer.

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Endocrine therapy resistance

As discussed above, endocrine therapy remains a crucial component of adjuvant breast cancer management in approximately 80% of patients presenting with ERα$^+$ tumors (114). Inhibiting the actions of ERα is accomplished indirectly through aromatase inhibitors that prevent the peripheral synthesis of estrogen, as well as directly through the use of SERMs, primarily tamoxifen. Tamoxifen exerts its effects by occupying the ligand binding domain of ERα, thereby inhibiting one of its two activation domains, AF2, which prevents estrogen-mediated transcriptional activation (115). Although the vast large majority of patients with ERα$^+$ tumors initially respond to tamoxifen treatment, nearly all patients who originally present with advanced disease, as well as a significant proportion of those who present with localized disease will ultimately progress to develop resistance to tamoxifen within 10 years (39). The mechanisms underlying this acquired resistance are multifactorial and include the phosphorylation and ligand-independent activation of ERα by EGFR and ERK1/2 (MAPK3/MAPK1), as well as the acquisition of ERα mutations that elicit its constitutive activation or enhanced recruitment of coactivators to increase signaling through ERα-dependent pathways (116). Furthermore, many patients demonstrate intrinsic resistance to tamoxifen, either by harboring primary tumors that do not express ERα, or by failing to respond to endocrine therapy despite possessing ERα$^+$ malignancies (117).

EMT programs have also been shown to play a crucial role in mediating both *de novo* and acquired resistance to tamoxifen. Indeed, tamoxifen resistant breast cancer cells exhibit mesenchymal-like phenotypes characteristic of post-EMT cells, including (i) downregulated expression of epithelial markers, such as E-cadherin and miR-27b, and (ii) upregulated expression of mesenchymal markers, such as TWIST1, SNAIL, Vimentin, N-cadherin, fibronectin, and ZEB2 (118–121). At present, the precise role EMT programs play in mediating tamoxifen resistance remains uncertain. For instance, it is unclear whether tamoxifen resistance drives breast carcinoma cells to undergo EMT or whether EMT programs precede and drive the development of tamoxifen resistance. With respect to the former supposition, the appearance of mesenchymal-like cells is linked to the activation of WNT signaling pathways only following initial acquisition of tamoxifen resistance. More specifically, tamoxifen-resistant post-EMT breast carcinoma cells exhibit significantly enhanced WNT3a and β-catenin signaling (**Fig. 1**), whose inactivation is sufficient in restoring epithelial phenotypes (122, 123). Similarly, intrinsic tamoxifen resistance that occurs in ERα$^-$ tumors has also been associated with EMT programs. For instance, ERα interacts with and enhances the activity MTA3 (metastasis associated 1 family member 3), a transcriptional corepressor that inhibits the expression of SNAIL and, consequently, prevents its ability to induce EMT programs (124). Thus, loss of ERα expression during the initiation of intrinsic tamoxifen resistance inactivates MTA3 and its suppression of SNAIL expression, resulting in the stimulation of EMT and invasion by developing mammary tumors (**Fig. 1**).

Alternatively, EMT programs can also act as instigating events that engender tamoxifen resistance. For example, overexpression of TWIST1 in MCF-7 cells resulted in a loss of ERα expression and tamoxifen resistance both *in vitro* and *in vivo*, events mediated by the binding of TWIST1 to inhibitory E-boxes within the ERα promoter (125). A similar loss in ERα expression was observed in MCF-7 cells engineered to overexpress SNAIL, leading to enhanced activation of the TGFβ pathways (126). Accordingly, the administration of neutralizing anti-TGFβ antibodies was sufficient to restore the antiproliferative activities of tamoxifen on xenografts derived from tamoxifen-resistant human breast carcinoma cells (127). Along these lines, EMT programs stimulated by TGFβ also elicit tamoxifen resistance by promoting receptor tyrosine kinase-dependent (e.g., EGFR and IGF1R) activation of SRC and MAPKs, which coalesce in mediating the cytoplasmic accumulation of ERα and its ability to engage in nongenomic signaling (128). Regardless of the cause and effect relationship between tamoxifen resistance and EMT programs, it is abundantly clear that endocrine resistance is highly associated with the invasive and metastatic characteristics afforded by EMT, as evidenced by the high degree of crosstalk between the effector pathways of EMT and tamoxifen resistance (**Fig. 1**).

The relationship between EMT programs and tamoxifen resistance in ERα$^+$ tumors also extends to BCSCs. For instance, chronic tamoxifen administration selects for a population of chemoresistant cells that express elevated levels of pluripotency-associated transcription factors, including SOX2, NANOG, and Oct4 (POU5F1; refs. 129, 130). Interestingly, BCSCs may in fact possess an inherent resistance to tamoxifen and other endocrine agents, a phenomenon reflecting the fact that normal mammary stem/progenitor cells exhibit a basal phenotype and lack expression of ERα. Thus, while recent evidence indicates that BCSCs likely originate from luminal epithelial cells (131), these transformed stem-like cells nevertheless retain features inherent in their normal physiologic counterparts, including the natural predisposition for being ERα$^-$ and thus unresponsive to tamoxifen (132). Mechanistically, elevated Notch signaling, particularly that mediated via NOTCH4, appears to be a critical component of BCSC maintenance in multiple models of breast cancer (133, 134). Accordingly, tamoxifen-resistant breast cancer cells exhibit increased levels of NOTCH4 and its activated targets, and as such, inactivating NOTCH4 mitigates the BCSC population in these resistant cells (119, 135).

Finally, the WNT signaling axis represents a unifying pathway operant in linking EMT and BCSCs to tamoxifen resistance (**Fig. 1**). Thus, besides the aforementioned studies that associated EMT-dependent chemoresistance to enhanced WNT signaling, Piva and colleagues (136) performed global gene expression analysis on BCSCs isolated from tamoxifen-resistant MCF-7 cells and observed extensive upregulation of WNT signaling effectors, including WNT3A and its receptor FZD4, as well as SOX2. Importantly, targeted inhibition of WNT signaling via administration of the small-molecule inhibitor against PORCN, IWP-2, not only restored tamoxifen sensitivity, but also significantly reduced the BCSC population (136). Taken together, these findings suggest that clinical targeting of WNT signaling may prove to be beneficial in abrogating tamoxifen resistance by inhibiting both EMT and CSC phenotypes.

Trastuzumab resistance

As discussed previously, the development of the HER2-targeting mAb trastuzumab revolutionized the treatment and clinical outcomes for breast cancer patients harboring aggressive HER2-amplified tumors. All too often, however, the vast majority of HER2$^+$ tumors will ultimately develop resistance to trastuzumab within 2 years (137, 138), an untoward event reflecting the emergence of EMT and CSC phenotypes. Interestingly, amongst the diverse collection of HER2-amplified breast cancer cell lines (139), those that display mesenchymal phenotypes are intrinsically resistant to the cytotoxic effects of trastuzumab as compared with their epithelial counterparts, a property reliant upon the upregulated expression of the master EMT transcription factor, SNAIL2 (140). Similarly, the acquisition of EMT phenotypes in HER2-overexpressing breast cancer cells in response to either TGFβ administration or expression of constitutively active TGFβ type I receptors (TGFBR1) dramatically reduced trastuzumab

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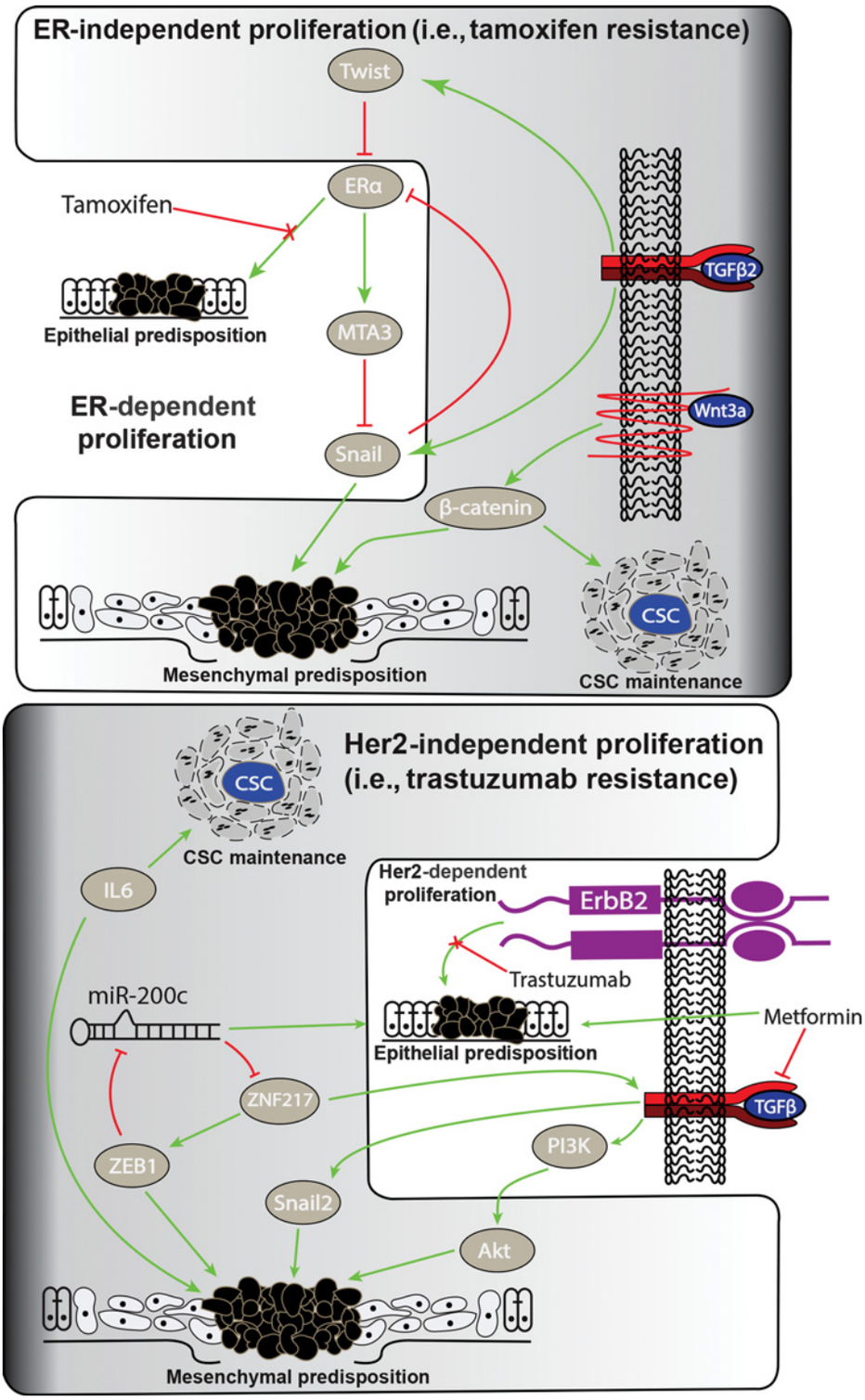

Figure 1.

Schematic depicting diverse role of EMT programs in driving tamoxifen (top) and trastuzumab (bottom) resistance. Prior to the development of tamoxifen resistance, luminal breast cancers are reliant upon ERα for their proliferative advantage while exhibiting epithelial phenotypes. The appearance of tamoxifen resistance correlates with the acquisition of mesenchymal features, thus promoting proliferation that is independent of ERα (shaded box; top). EMT programs also elicit tamoxifen resistance, particularly that induced by TGFβ, SNAIL, and TWIST1. Conversely, acquired resistance to tamoxifen can also compel cells to undergo an EMT program. Loss of ERα expression not only abolishes the effectiveness of tamoxifen, but also induces EMT via loss of MTA3 expression that relieves its suppressive effects on SNAIL. Similarly, cells engineered to be resistant to the growth inhibitory effects of tamoxifen rely heavily upon WNT3a/β-catenin pathways, a trait shared by cells that have undergone EMT. In addition, WNT3a signaling is crucial in maintaining the pool of tamoxifen-resistant BCSCs. Resistance to trastuzumab equally relies on EMT-dependent pathways, as $Her2^{+}$ tumors are marked by a mesenchymal/basal phenotype upon acquiring resistance to trastuzumab (i.e., Her2-independent growth; shaded box; bottom). TGFβ induces EMT and trastuzumab resistance through SNAIL2 and PI3K/AKT-dependent pathways, while a downregulation of miR-200c promotes trastuzumab resistance and a mesenchymal phenotype, two effects that are reverted by reestablishment of miR-200c levels. IL6 plays a unifying role in Her2-independent proliferation, as the cytokine influences both the stemness and epithelial/mesenchymal status by expanding the BCSC population via EMT. Metformin treatment restores both an epithelial phenotype and subsequent sensitivity to trastuzumab.

sensitivity in part via activation of PI3K/AKT-mediated pro-survival pathways (**Fig. 1**; ref. 141). Furthermore, restoring miR-200c expression in trastuzumab-resistant SKBR-3 cells resulted in a mesenchymal-to-epithelial transition (MET), and consequently, in the resensitization of SKBR-3 cells to trastuzumab. Mechanistically, these events reflected the ability of miR-200c to downregulate the expression of ZNF217, a transcriptional activator of the EMT regulators TGFβ2, TGFβ3, and, importantly, ZEB1, a potent transcription factor that drives mesenchymal phenotypes in part by negatively regulating miR-200c itself (**Fig. 1**; refs. 142, 143). Collectively, these studies highlight the

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permissive function of EMT programs in promoting the development of trastuzumab resistance in HER2$^+$ breast cancers.

It is interesting to note that the actions of HER2 have also been implicated in influencing the "stemness" of both normal and malignant mammary stem cells. For example, engineering nontumorigenic MECs to overexpress HER2 readily expanded an ALDH$^+$, stem-like population that was highly proficient in mammosphere formation; they also harbored transcriptional profiles associated with stem-like states (144). Along these lines, mesenchymal and stem-like phenotypes play crucial and seemingly inseparable roles in eliciting trastuzumab resistance. Indeed, this situation is further exemplified in patients with HER2$^+$ breast cancer, of which more than 40% will develop trastuzumab-resistant tumors that also harbor inactivating mutations in the tumor suppressor, PTEN (145). Although PTEN mutations typically result in the activation of AKT and its stimulation of cell-cycle progression (146), recent studies indicate that loss of PTEN unites EMT programs with BCSCs as they acquire chemoresistant phenotypes. For instance, HER2$^+$ breast cancers lacking PTEN function rely upon the actions of the inflammatory cytokine IL6, which influences both the plasticity and stemness of these developing carcinomas (**Fig. 1**). Indeed, the emergence of trastuzumab resistance in HER2$^+$ breast cancer reflects a significant increase in IL6 production that promotes the expansion of BCSCs (i.e., CD44$^+$/CD24$^-$ phenotype) and activation of EMT-based gene expression signatures. Importantly, these oncogenic events were readily prevented by administration of neutralizing IL6 antibodies (147). Finally, prolonged exposure of PTEN-deficient HER2$^+$ cells to trastuzumab not only expanded the pool of BCSCs, but also enabled ER$^+$/PR$^+$/HER2$^+$ cells to assume a TNBC phenotype complete with heightened chemoresistant characteristics (148).

Doxorubicin resistance

Doxorubicin is an anthracycline antibiotic that elicits cytotoxic effects by intercalating into DNA, thereby inhibiting the advancement of topoisomerase II and preventing the relaxation of supercoiled DNA. In doing so, doxorubicin readily induces single- and double-stranded DNA breaks that inactivate DNA replication and transcription (149). Although significant toxicities limit the therapeutic index of doxorubicin, it nevertheless remains an important component of therapeutic strategies for metastatic and advanced disease, especially in patients with TNBC that typically exhibit a 35% to 50% response rate (150, 151). As is too often the case, resistance to doxorubicin develops in a significant proportion of patients in a manner dependent upon EMT programs and BCSC phenotypes. Indeed, EMT programs and their induction of both SNAIL and TWIST1 can play a permissive role in the acquisition of resistance to doxorubicin, presumably via upregulation of P-glycoprotein (152). Similarly, SNAIL-mediated upregulation of PARP1 in human MDA-MB-231 breast cancer cells also contributes to doxorubicin resistance, an event that is mitigated by administration of PARP inhibitors, such as ABT-888 (153). Along these lines, EMT programs initiated by AXL also elicit resistance to doxorubicin, doing so by stimulating an AKT→GSK3B→β-catenin (CTNNB1) pathway coupled to the expression of ZEB1 (154).

Interestingly, the mechanisms underlying EMT-associated resistance to doxorubicin rely heavily upon the expression and activity of miRNAs. For instance, rendering MCF-7 cells resistant to doxorubicin significantly reduces the expression of miR-200c, an event associated with EMT programs and their propensity to downregulate the expression of E-cadherin and upregulate that of ZEB1. Restoring miR-200c expression in these doxorubicin-resistant MCF-7 cells induced a MET program that reinstated an epithelial-like phenotype and increased sensitivity to doxorubicin due in part to PTEN-dependent inactivation of AKT (155). Similarly, miR-644a targets C-Terminal Binding Protein 1 (CTBP1) to induce epithelial states in multiple breast cancer cell lines, ultimately sensitizing these cells to doxorubicin (156). Conversely, aberrant expression of oncomiRs has also been associated with the acquisition of doxorubicin-resistance. For example, the miR-106b∼25 cluster, which consists of miR-106b, miR-93, and miR-25, is upregulated in doxorubicin-resistant breast cancer cells and promotes doxorubicin resistance in minimally transformed MECs by depleting the expression of the histone acetyltransferase EP300, a positive regulator of E-cadherin expression (157). Indeed, targeted inactivation of both EP300 and E-cadherin was found to promote EMT and provoke doxorubicin resistance (155).

The expansion of BCSCs correlates significantly with the appearance of doxorubicin resistance. Indeed, upon acquiring resistance to doxorubicin, MCF-7 cells become enriched for EMT markers and a CD44$^+$/CD24$^-$ population, thus enhancing their tumor-initiating capacity and differentiation capabilities (158, 159). Moreover, reducing the expression of CD44$^+$ BCSCs dramatically increased their sensitivity to the cytotoxic effects of doxorubicin (160), lending support to the notion that stemness inherently initiates and maintains doxorubicin resistance. Along these lines, the inherent resistance of BCSCs to doxorubicin also reflects their quiescent behaviors, which renders BCSCs less susceptible to the cytotoxic activities of this DNA-damaging agent (161). Finally, *de novo* resistance of BCSCs to doxorubicin also reflects the upregulated expression of drug efflux pumps, particularly ABCC5 and P-glycoprotein that are highly effective at targeting doxorubicin (158, 159, 162).

Similar to their convergent roles in mediating trastuzumab resistance, BCSCs and EMT once again exhibit overlapping functions to elicit doxorubicin resistance. For instance, administration of a TGFβ type I receptor inhibitor (i.e., [3-(pyridine-2yl)-4-(4-quinonyl)]-1H pyrazole; ref. 163) to 4T1 cells was sufficient to prevent their initiation of EMT programs in response to doxorubicin, resulting in (i) contraction of the BCSC pool and diminished mammosphere-forming activity; and (ii) enhanced cytotoxic activity of doxorubicin against 4T1 cells (164). Similarly, inducible expression of miR-220c in developing claudin-low breast tumors in mice promoted a more epithelial-like state that diminished the tumor-initiating capacity of these cells, as well as augmented their sensitivity to doxorubicin (165). Collectively, these studies highlight important and clinically significant links between EMT programs and CSCs during mammary tumor progression and the acquisition of doxorubicin resistance.

Taxane resistance

Paclitaxel and docetaxel are β-tubulin (TUBB)-binding drugs that have been widely deployed against both early-stage and metastatic breast cancers since the 1990s, owing, in part, to the fact that these compounds are amongst the most active cytotoxic agents against breast cancer. Paclitaxel is isolated from leaf extracts of the Pacific Yew tree, while docetaxel is a semisynthetic analogue of paclitaxel. Mechanistically, both compounds function to disrupt microtubule formation that elicits G_2–M arrest and mitochondrial-mediated apoptosis (166). Moreover, combining taxanes with anthracycline-based treatment regimens significantly improves both disease-free and overall survival rates of patients with breast cancer, particularly those with early-stage disease and independent of common risk factors, such as hormone receptor expression, nodal status, and patient age (167). Despite the remarkable effectiveness of taxanes in targeting breast cancers, resistance to these cytotoxic agents ultimately develops in the vast majority of patients with breast cancer. Interestingly, acquired

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resistance to taxanes in human breast tumors (168) and established cell lines (169, 170) is associated with the appearance of EMT phenotypes. In fact, comparing biopsies obtained both before and after anthracycline and taxane (i.e., either paclitaxel or docetaxel) regimens demonstrated significant upregulation of TGFβ and BCSC gene signatures in chemotherapy-treated tumors. Importantly, administration of the TGFβ type I receptor inhibitor, LY2157299 suppressed the ability of paclitaxel to induce IL8 expression and its induction of BCSC self-renewal and expansion; this treatment regimen also prevented disease recurrence in mice harboring SUM159 tumors (168). Thus, these findings support the notion that inactivating EMT programs driven by TGFβ may provide new inroads to alleviate taxane resistance in clinical settings.

Additional evidence linking EMT programs to taxane resistance was obtained by isolating highly invasive variants from their poorly invasive counterparts. In doing so, highly invasive breast cancer cells exhibited EMT phenotypes dependent upon the activation of a TWIST1→AKT2 signaling axis. Moreover, this emergent EMT population of cells was also resistant to paclitaxel, an event that could be neutralized by genetic inactivation of AKT2 in TWIST1-expressing breast cancer cells (171). Along these lines, TNBC resistance to docetaxel reflects the concomitant activation of the Notch pathway, resulting in the activation of SNAIL- and SLUG-dependent EMT programs. As above, preventing EMT programs by administration of the γ-secretase inhibitor, PF-03084014 restored the cytotoxic activities of docetaxel to TNBCs, as well as reduced the self-renewal capacity of their BCSCs (172). Similar findings pertain to targeted inactivation of (i) AXL in post-EMT TNBCs, an event that synergizes with docetaxel to suppress the growth of MDA-MB-231 tumors in mice (173); and (ii) SKP2 in post-EMT luminal and $HER2^+$ breast cancers, leading to their enhanced sensitivity to paclitaxel (170). Collectively, these studies highlight important and clinically significant links between EMT programs and CSCs during mammary tumor progression and the acquisition of taxane resistance.

Therapeutic Targeting of EMT and Stemness

The convergent roles played by BCSCs and EMT programs during the development of drug resistance offer a plethora of potential molecular targets whose modulation could aid in overcoming the growth and relapse of various breast cancer subtypes. For example, an encouraging prospective pharmacologic modifier of EMT- and BCSC-dependent trastuzumab resistance in $HER2^+$ lesions appears to be the drug metformin, a biguanide with expansive clinical utility as a first-line therapy for type II diabetics (**Fig. 1**). Recent studies revealed that single-agent metformin treatment of trastuzumab-resistant breast cancer cells specifically targets BCSCs by reducing the proportion of $CD44^+/CD24^-$ cells and their capacity to form mammospheres, thereby inhibiting the growth of trastuzumab-resistant xenografts (174). Importantly, the anticancer activities of metformin were enhanced synergistically by administration of trastuzumab, indicating that metformin can restore tumor sensitivity to trastuzumab. Consistent with the ability of EMT programs to generate BCSCs, it is not surprising to find that metformin can also suppress EMT and can induce MET in a variety of cancers, including breast, lung, and prostate (175–177). Future studies need to address the broader impact of metformin and the mechanisms whereby it overcomes treatment refractory disease in patients with recurrent $HER2^+$ tumors.

A defining feature of the development of chemoresistance in breast carcinomas is the necessity of neoplastic cells to exist in a plastic cellular state. Such a malleable phenotype allows breast cancer cells to adopt adaptive signaling programs that allow them to efficiently respond to microenvironmental cues, including pharmacologic insults, to facilitate their survival and outgrowth (5). Similarly, the acquisition of stem-like phenotypes is characterized by the necessity of transformed cells to persist in a malleable state, as CSCs must possess a degree of plasticity that allows for their differentiation into the bulk population of a tumor. Moreover, it is now clear that non-CSCs can readily undergo genetic and epigenetic alterations that bestow them with tumor-initiating properties (178–180), indicating that CSC hierarchies do not represent unidirectional transformations of cellular phenotype.

Importantly, the phenotypic interconversions that define cellular plasticity primarily take place without concomitant genetic changes (i.e., they are epigenetic in origin). The epigenetic landscape of a cell is controlled by intrinsic and extrinsic factors that dictate the extent and location of dynamic DNA and histone modifications (e.g., methylation and acetylation), culminating in a steady state equilibrium of nongenomic alterations that interact with the genomic background to bring malignant cells to a distinct point on a continuum of potential cellular phenotypes. It is therefore unsurprising that breast cancer cells rely heavily upon alterations in their epigenetic landscape as they undergo transformations along their phenotypic spectrum that confer chemoresistance and stemness (181–184). Importantly, pharmacologic inhibition of the chromatin remodeling that confers chemoresistant traits to breast cancer cells (i.e., BET inhibition via JQ1 administration) is capable of preventing the acquisition of a drug-resistant state (184). Targeted modulation of the molecular mediators of epigenetic plasticity is therefore a compelling strategy aimed at preventing the acquisition of stem-like and chemoresistant traits during the natural course of breast cancer progression.

Accordingly, alleviating therapeutic resistance in breast cancers will require not only the complete eradication of chemoresistant BCSCs, but also the means to prevent their plasticity via cycles of EMT-MET programs. Along these lines, a high-throughput drug screen against mesenchymal-like BCSCs identified the antimicrobial and highly selective potassium ionophore salinomycin as an agent that possesses selective cytotoxicity against mesenchymal BCSCs, including those resistant to standard-of-care breast cancer chemotherapies (**Fig. 2**; ref. 185). The cytotoxic activity of salinomycin against BCSCs depends upon multiple mechanisms, including (i) elevated DNA damage and cell-cycle arrest (186); (ii) enhanced modulation, both positively and negatively, of autophagy (187); and (iii) diminished WNT/β-catenin signaling that results in the suppression of P-glycoprotein expression (188, 189). Beyond its preclinical successes, salinomycin has also generated excitement in clinical settings, as multiple case studies have demonstrated regression of metastatic breast and vulvar cancer lesions in salinomycin-treated patients that had exhausted all alternative therapeutic strategies (190).

Finally, it is important to note that chronic administration of salinomycin to several breast cancer cell lines results in the emergence of resistance to this antimicrobial. Interestingly, salinomycin-resistant breast cancer cells appear to have undergone a MET program, as they are highly epithelial in nature and appear hypersensitive to standard-of-care chemotherapies, including doxorubicin (**Fig. 2**; ref. 191). As such, these findings suggest the potential use of salinomycin during neoadjuvant or inductive therapy settings as a means to eliminate post-EMT, mesenchymal-like CSCs, followed by administration of conventional therapeutic regimens to target the surviving population of post-MET, epithelial-like CSCs responsible for disease recurrence (**Fig. 2**). Along these lines, attempts to identify compounds capable

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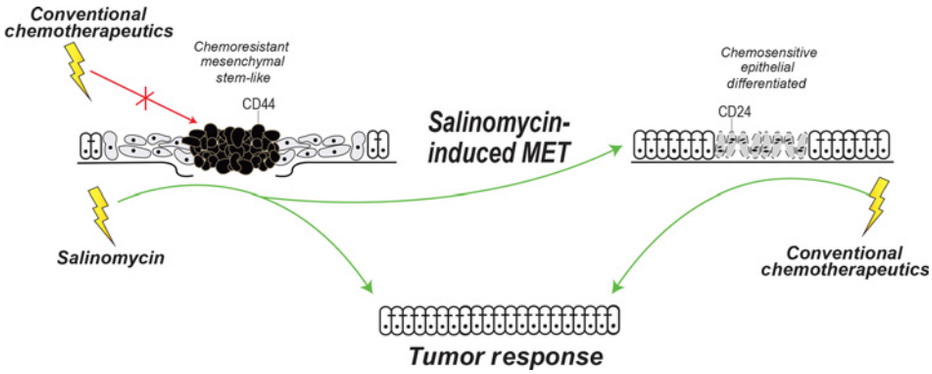

Figure 2.

Role for salinomycin in overcoming breast cancer therapeutic resistance. Breast cancer cells undergoing EMT or those associated with stem-like states are frequently resistant to standard-of-care breast cancer therapies. Salinomycin has shown clinical utility in selectively targeting chemoresistant, mesenchymal BCSCs. However, breast cancer cells can develop resistance to single-agent salinomycin. Nonetheless, the emergent salinomycin-resistant population is highly epithelial in nature [i.e., has undergone mesenchymal–epithelial transformation (MET)] and is resensitized to commonly used chemotherapeutics.

of inducing MET programs as a means to restore breast cancer sensitivity to standard-of-care agents have recently discovered that forskolin-mediated activation of PKA (PRKAC) could induce MET programs, thereby inhibiting the oncogenic activities of BCSCs both *in vitro* and *in vivo*. Moreover, pharmacologic activation of PKA also sensitized breast cancer cells to chemotherapy regimens (192, 193), thus highlighting the potential of harnessing and manipulating the inherent plasticity of breast BCSCs in clinical settings.

Conclusions and Future Directions

Defining the molecular mechanisms that underlie the initiation of EMT programs across genetically distinct breast cancer subtypes remains an important and unanswered question concerning the development, progression, and recurrence of this deadly disease. Indeed, among the spectrum of EMT-associated properties, those linked to the acquisition of chemoresistant and BCSC phenotypes likely play a disproportionate role in contributing to the mortality of breast cancers. As such, developing novel agents capable of targeting EMT programs will provide unique therapeutic windows aimed at eliminating BCSCs by preventing their expansion and self-renewal following induction of MET programs. Cumulatively, effective inactivation of EMT programs will also alleviate disease recurrence and restore sensitivity to standard-of-care chemotherapies, thereby promoting durable complete pathological responses in patients with late-stage disease. *In vivo* post-EMT cells are generally nonproliferative, a property that contributes to their insensitivity to chemotherapies that specifically target cell proliferation or DNA damage pathways. Thus, driving breast cancer cells to exit EMT programs may reactivate proliferative programs, further sensitizing recurrent tumors to traditional standard-of-care agents. Future studies need to address these possibilities, as well as explore the potential of deploying EMT inhibitors as a means to alleviate immune tumor tolerance, thereby enhancing the effectiveness of immune checkpoint inhibitors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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