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Targeting Breast Cancer Metastasis

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ABSTRACT: Metastasis is the leading cause of breast-cancer-associated deaths. Despite the significant improvement in current therapies in extending patient life, 30–40% of patients may eventually suffer from distant relapse and succumb to the disease. Consequently, a deeper understanding of the metastasis biology is key to developing better treatment strategies and achieving long-lasting therapeutic efficacies against breast cancer. This review covers recent breakthroughs in the discovery of various metastatic traits that contribute to the metastasis cascade of breast cancer, which may provide novel avenues for therapeutic targeting.

KEYWORDS: breast cancer, metastasis, metastatic traits, targeted therapy, precision medicine

Introduction

Breast cancer is the most common cancer among females worldwide, with an incidence rate of over 1.6 million cases per year.¹ Because of the significant advancements in diagnosis, therapy, and disease prevention over the past 50 years, breast cancer, unlike many other lethal cancers, is now considered a manageable disease. The five-year survival rate is up to 99% if the tumors are diagnosed early, and many patients are free of the disease for their lifetime. However, we remain far from a world without breast cancer, as it causes more than 0.5 million deaths every year. Over 90% of these patients die of metastasis, which is when cancer cells depart from their tumors of origin, spread systematically, and colonize at distant organs.² These metastatic lesions invade vital organs and deteriorate the patient’s health, forming multiple foci that are challenging to surgically remove and developing resistance to the systematic therapies that are currently available. Consequently, combating metastasis is of core importance in winning the war against breast cancer.

Genomic Landscape of Breast Cancer

A comprehensive portrait of the genomic makeup of breast cancer has now become available as a result of multiple high-throughput profiling efforts.³⁶⁷ These genomic data provide novel insights on the etiology of breast cancer. Breast cancer is traditionally classified into three subtypes based on the receptor status of estrogen receptor (ER), progesterone receptor (PR), and oncogene ERBB2 (HER2), termed ER+, HER2+ (ER+/PR+/HER2+), and triple-negative (TN) (ER−/PR−/HER2−). These receptor subtypes have been further refined into five intrinsic breast cancer subtypes based on a 50-gene expression classifier (PAM50).⁸ These distinct molecular subtypes differ in their disease progression, patterns of metastatic spread, clinical prognosis, and response to therapy. Thus, they have important implications on patient stratification, treatment planning, and clinical management for breast cancer.⁹

ER+ tumors, also referred to as luminal (LUM) subtypes by expression subtyping, account for ~70% of breast cancer cases. These tumors typically express high levels of ER and PR, and show an ER-dependent growth phenotype. These tumors are sensitive to estrogen withdrawal and are treated by hormonal therapy.¹⁰ ER+ tumors can be further divided into LUM-A and LUM-B subtypes based on the PAM50 classifier. LUM-B tumors tend to have higher tumor grades and poorer prognosis than the LUM-A tumors, and they are often also HER2+, Ki67+ (a cell proliferation marker), or PR low.⁴ The HER2 subtype, constituting 10–15% of all breast cancer cases, is driven by the receptor tyrosine kinase (RTK) oncogene ERBB2. These tumors are characterized by ERBB2 gene amplification/overexpression and responsiveness to anti-HER2 therapy, and are managed with this treatment modality in the clinic. TN breast cancers (15–20%), often...
equated to basal-like breast cancers by expression subtyping, represent the most aggressive breast tumors among the three subtypes. There is no targeted therapy currently available, and patients are mainly managed with cytotoxic chemotherapy. A further refined classifying method, based on consensus of copy number and expression patterns, has been proposed to divide breast cancer into 10 subtypes. The integration of this novel method with the established classification systems and its clinical significance remains to be further assessed.

Breast cancer has intermediate genomic alteration complexity and global mutation frequency among all cancer types. ER+ tumors are the quietest in genome-wide alterations among the three subtypes, but they harbor the most recurrently mutated genes, including activating mutations for PI3 kinase (PI3K) catalytic subunit PIK3CA and loss-of-function mutations for tumor suppressor TP53, mammary lineage specifiers GATA3, FOXA1, p38/JNK1 stress pathway kinases MAP3K1 and MAP2K4, and cell adhesion molecule CDH1. Notably, the estrogen receptor gene ESR1 itself is rarely mutated or amplified in breast cancer. This raises an interesting question of whether ER overexpression is a driver event of ER+ tumor transformation. In normal breast, ER expression is restricted to a small subset of quiescent LUM cells within the mammary epithelium, whereas in cancerous lesions, elevated ER expression is detected in a large proportion of proliferating cells. How ER expression is elevated in ER+ tumors remains poorly understood and needs to be further investigated.

HER2 subtype is dominated by ERBB2 amplification. A substantial proportion of these tumors also harbor TP53 or PIK3CA mutations. Mutations in other genes are seen at a much lower frequency (such as PIK3R1, 4%). TN breast cancers, in contrast, are marked by the mutations in the tumor suppressor TP53 (up to 80%). Consistent with the loss of this gatekeeper in genomic integrity and DNA repair, TN breast tumors typically display more genomic chaos, enrichment in BRCA1 mutation, and sensitivity to chemo reagents. No notable oncogene mutations are recognized in this subtype, except PIK3CA at a low frequency (9%). This is in line with the idea that heterologous oncogenic signaling may be able to drive tumorigenesis in this subtype and that TN breast cancer as a whole is a heterogeneous group. More refined subgroups perhaps exist within this subtype.

A recurrent theme from these genomic characterizations is the activation of PI3K–Akt signaling, seen in all three breast cancer subtypes. In ER+ breast cancer, PIK3CA mutation itself accounts for 40% of cases. The rest of the cancers may reach PI3K–Akt activation through loss or downregulation of the negative regulators Pten or INPP4B, through copy number amplification or overexpression of other RTKs such as IGF1R or FGFR, or through active ER signaling itself. In HER2+ breast cancer, PI3K–Akt activation is a major downstream event of ERBB2-initiated oncogenic signaling. In TN breast cancer, even though PIK3CA mutation is seen at a low rate, the PI3K pathway activity inferred from gene expression or protein array signatures is actually the highest. This pathway may be activated in these tumors through PIK3CA copy number gain, Pten or INPP4B loss, EGFR amplification or overexpression, or Akt3 amplification. Viewed in this way, Akt activation seems to be a hallmark of breast cancer. Indeed, deregulated PI3K–Akt signaling also plays a pivotal role in many aspects of breast cancer metastasis (as discussed below).

It is important to note, however, that most of these genomic studies are based on primary tumors and not metastasis samples. Thus, the amount of information one can deduce for metastasis biology and relate it to therapeutic intervention remains to be seen.

Metastatic Patterns of Breast Cancer

It is well known from clinical observations that different tumor types display distinct organ tropisms in metastatic patterns. Breast cancer displays distinct tropisms depending on the subtypes. Bone, lung, liver, and brain are the common target organs for breast cancer metastasis, in addition to distant lymph nodes. ER+ tumors have the best prognosis with a low incidence rate within the first five years. But this rate gradually increases as the time extends beyond five years (up to 40%). Bone is the predominant metastatic site, whereas brain is much less affected. In contrast, TN breast tumors display the worst prognosis, with a spiking incidence rate within the first one to two years and virtually all metastases occurring within the first five years. Visceral organs, including brain and lung, are more frequently affected in TN tumors. HER2+ tumors are also considered an aggressive disease. With the invention of anti-HER2 therapy, the prognosis has been much improved and patient lifespan is significantly prolonged. The therapy is quite effective in controlling extracranial lesions but leave brain metastasis a remaining challenge.

What underlies the metastasis tropism has been a heated topic. The spreading pattern of blood flow can explain some tumor types. For example, the primary site of colon cancer metastasis is the liver and the second site is the lung. This is explained by the massive cell trapping in the liver capillary after mesenteric circulation and then in the lung after cancer cells come out of circulation from liver. However, such an explanation is not likely to be applicable in the case of breast cancer. An alternative view to the circulation pattern was first proposed by Paget in the 19th century, who posited that disseminated cancer cells (seeds) can form metastases as they reach a microenvironment (soil) that is congenial enough for their survival and proliferation. This seed-and-soil hypothesis has received extensive support with the identification of gene mediators that contribute to metastasis formation.

Even though it remains unclear what determines the “seeds” will fit in a particular soil, some basic nature of the
breast cancer subtypes may shed light on their organ preferences. Bone is a major target site for ER+ breast cancer metastasis. It is worth noting that bone is also rich in estrogen, which, in normal physiology, plays a critical role in the maintenance of bone homeostasis and remodeling. Therefore, ER+ tumor cells lodging in the bone marrow may have a particular advantage in hijacking the available estrogen for proliferating signal. HER2+ tumors are driven by the ERBB2/ERBB3 heterodimer in a ligand-independent manner and are self-sufficient for oncogenic signaling. This may give them a particular advantage for developing brain metastasis, because the brain has fewer immune cells compared to the rest of the body, and all it may require is self-sufficiency in proliferation. One may also speculate that the extensive metastatic potential and overall aggressiveness of the TN breast cancers are related to their high activity of PI3K–Akt signaling and cell viability, though this requires further experimental validation.

Metastasis Cascade
Cancer cells need to undergo a series of steps in order to depart from the primary site and spread to various organs. This metastasis cascade comprises the steps of cancer cells: (1) becoming locally invasive and migratory, (2) reaching the blood vessel and intravasating into the circulation, (3) circulating via the blood flow, (4) arresting and extravasating to the distant organ, (5) surviving the initial hostile stress, and (6) reinitiating outgrowth and co-opting the distant stroma (Fig. 1).

To achieve each of these steps, cancer cells have to encounter multiple layers of natural barriers and challenge the defined organization and the established homeostasis of target organs. As a result, one could imagine that metastasis is an extraordinarily inefficient process. It is estimated that more than 99.98% of disseminated cancer cells die before a metastasis could form. However, early steps of metastasis, such as intravasation and extravasation, can occur surprisingly more efficiently than expected. Tumors at early stages may already release cancer cells into circulation before signs of invasion. Experimental evidence showed that more than 80% of inoculated cells succeed in extravasating. Even normal epithelial cells may have the ability to invade distant organs. Most attrition occurs after the extravasation step. Less than 3% of the cells can survive to form micrometastases and an even lower percent makes to proliferate and forms macro lesions. Thus, it is more and more recognized that survival and outgrowth in foreign tissue are the rate-limiting steps for metastasis colonization and constitute the bottleneck of the metastasis cascade.

Local Invasion
To overcome the multiple organismal barriers, tumor cells have to gain an extra set of gene activities or characteristics, in addition to their ability to grow without restriction in the primary tumor. Epithelial-to-mesenchymal transition (EMT) governs the local invasion step of epithelial cancer. Tumor cell properties, such as becoming migratory and secreting extra cellular matrix (ECM)-destructing enzymes, are associated with this program. EMT is controlled by a transcriptional program comprising Twist, Slug, Snail, ZEB1, ZEB2,
and miR-200s. These factors respond to external stimuli, such as TGFβ, WNT, or hypoxia, and act in concert to elicit changes in the surface molecule profiles, including loss of E-cadherin and gain of N-cadherin or vimentin, transition from CD44lowCD24high to CD44highCD24low, and expression of matrix metalloproteinases (MMPs). These traits collectively lead cells to invade the surrounding stroma, reach the blood vessel, and intravasate into the circulation.

**Circulation**

To survive the transit in circulation, cancer cells need to resist anoikis, shear forces of the blood flow, and innate immune attack. Anoikis is a death stress for epithelial cells from attachment. It has been shown that circulating tumor cells (CTCs) overexpress TrkB or WNT2 to resist this form of death stimulus. CTCs also form clusters with platelets, termed emboli, to evade killing from shear forces as well as predation of natural killer (NK) cells. It is noteworthy, however, that circulation is a relatively quick process and cells may reach other organs within minutes after entering circulation. This short period in transit may partially explain why the attrition of cells during circulation is not significant.

**Extravasation**

Extravasation and intravasation are, to some degree, mirrored processes. Thus, genes contributing to extravasation steps may also participate in the extravasating process. Epiregulin (EREG), MMP-1, MMP-2, prostaglandin-endoperoxide synthase 2 (PTGS2, also called cyclooxygenase-2 (COX-2)), and fascin (FSCN) are genes involved in vessel remodeling and intravasation, and they also facilitate disruption of vascular junctions and let cancer cells invade into distant organs.

Moreover, the normal vascular organization of a distant organ may differ from that of primary tumors and require additional genes for penetrating distant tissues. The tight lung capillaries require the attainment of angiopoietin-like 4 (ANGPTL4) in cancer cells for the opening and leakage of pulmonary vessels. The even tighter structure of blood–brain barriers, composed of endothelial cells, pericytes, and astrocytes, requires additional players such as α2,6-sialyltransferase (STALNAC6) and heparin-binding epidermal growth factor (HB-EGF) for extravasating into the brain.

**Survival**

After passing these steps, the disseminated tumor cells (DTCs) face the biggest hurdle toward their colonization, the foreign microenvironment. The differences in stromal components, tissue organization, matrix composition, and cytokine environment all impose immense threats on the just arrived cancer cells. As a result, the limited prosurvival signal that cancer cells can grasp from surroundings may play a critical role in determining whether they can take a foothold. The genes required for survival in different sites also differ as the organ varies. When breast cancer cells lodge in the bone marrow, cancer cells take advantage of CXCL12 and IGF1 from the surrounding stroma to induce PI3K–Akt survival signaling. Src amplifies the magnitude of this signaling and contributes to cancer cell survival in bone metastasis. In the case of lung metastasis, by bridging with macrophages, VCAM-1 in breast cancer cells elicits the activation of PI3K–Akt survival signaling and confers cell fitness. In the brain, cancer cells protect themselves by secreting serpins, thus shielding themselves from being killed by the reactive brain stroma.

**Outgrowth**

The surviving cancer cells then need to engage extra genes that could modify the distant stroma and extract signals that trigger their intrinsic oncogenic signaling for proliferation. Bone is well investigated in this respect. Bone metastatic breast cancer cells express a set of pro-osteolytic genes to tip the intricate balance between osteoblasts and osteoclasts. These genes, whether working through osteoblasts (parathyroid hormone-related protein (PTHrP), jagged1 (JAG1) or through osteoclasts (interleukin-8 (IL-8), interleukin-11 (IL-11), osteopontin (OPN), A disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1), MMP-1) lead to activation of the osteolytic cycle. The activated osteoclasts dissolve the bone matrix and let it release the growth factors deposited within. These factors foster the cancer cell growth and exacerbate the feed-forward interaction between cancer cells and bone stromal cells, resulting in a vicious cycle and patient symptoms, including bone pain and fracture. In pulmonary metastasis, CXCL1 secreted by lung-disseminated cancer cells recruits myeloid progenitors, which in turn provides S100A/B for cancer cell proliferation. Such paracrine interactions are also evidenced between cancer cells and other resident or recruited stromal components, including macrophages and endothelial cells. Cancer cells engage the ECM proteins tenascin C (TN-C) and periostin (POSTN), either from their own source or from the surrounding stroma, to create a stem-like niche that potentiates WNT and NOTCH signaling for growth in the lung. What program contributes to the propagation of brain metastases is less understood. Recent work suggests that juxtacrine interaction between cancer cells and astrocytes may provide such an advantage. The brain microenvironment may also elicit reprogramming of cancer cells toward neuronal characteristics to adapt to the brain stroma.

Collectively, completion of the metastasis cascade requires coordinated action of multiple gene programs, and distinct tissue microenvironments require distinct gene sets for organ-specific colonization. These genes either overcome or compensate for the incompatibilities between the intrinsic growth demands of the seeds and the extrinsic restrictions imposed by the particular foreign soil.

**Evolution of Metastatic Traits**

How metastatic traits arise as the primary tumor evolves remains a poorly investigated question. Identifying the
drivers of metastasis is of great interest, as they may be key targets for therapeutic intervention. Additionally, these drivers may serve as indicators of metastatic risk that could play an important role in stratifying breast cancer patients for adjuvant therapy. A clonal view of tumor evolution implicates that the genetic and epigenetic instabilities that are intrinsic to cancer cells generate sufficient diversity among the cancer cell population, upon which the selective pressure acts to foster the expansion of favorable clones. Such cycles of mutation and clonal selection drive the expansion of the fittest tumor cells and lead to the emergence of various traits in the tumor. This Darwinian model is well exemplified by the sequential progression of colorectal cancer. The serial transition from a benign tissue to an adenoma and to an aggressive carcinoma is a result of multiple waves of clonal expansion driven by mutations in APC, KRAS, PIK3CA, TP53, and TGFβ pathways. The study of these tumor drivers led to further speculation as to whether metastatic traits are also driven by additional mutations. Rare clones possessing such mutations from primary sites may be selected and expanded as they spread and reach secondary sites. If these speculations were true, one would expect that the molecular determinants of metastasis would not be overtly manifested in the bulk primary tumor but be specific to metastases, because the distinct microenvironments of the distant organs and the primary sites impose different selective pressures on tumor cells. As a result, metastases and their primary tumors should be sufficiently different in both genomic composition and expression. However, pair-wise comparisons of primary tumors and metastases in multiple tumor types, including breast cancer, failed to find mutations that were private, specific, and recurrent to metastases. On the contrary, the identified genetic alterations in metastases are also commonly shared by the primary tumor. Transcriptomic profiling of tumor and metastasis pairs also showed that the two lesions are more alike than different. Given that these findings need to be confirmed by larger scale studies, no driver mutations that are specific for metastasis have been identified to date. Furthermore, gene activities contributing to distant metastasis can already be detected in primary tumors. As an ensemble, they form gene signatures that predict the likelihood of metastasis and patient prognosis. As a result, the clonal view of tumor evolution seems to contradict the actual failure in identifying metastasis specific driver mutations. The Darwinian selection of rare clones also finds itself incompatible with the clinical observation of metastatic mediators being expressed in large segments of primary tumors. These apparent discrepancies lead to the conundrum as to how metastatic traits, especially those that are distant organ specific, could ever be evolved in the primary tumor.

These conceptual inconsistencies and confusions start to become solved by recent insights on the evolution of metastatic traits in breast tumors. A case in point is Src activity in breast cancer cells, which is both a functional mediator and a prognostic indicator of bone metastasis in breast tumors. Querying how Src activity is enriched in tumors, three general principles emerge that may lead to the evolution of metastatic traits.

**Oncogenically Encoded**

As evidenced in ER+ breast tumors, in some cases, the metastatic traits are encoded in the oncogenic signaling and are intrinsic to the tumor cell properties. ER signaling biochemically activates Src and directly confers ER+ tumor cells high Src activity. Src contributes to bone metastasis and promotes cancer cell survival by amplifying the magnitude of PI3K-Akt signaling induced by CXCL12 and IGF1, the mesenchymal cytokines from the bone marrow. Similarly, E-cadherin expressed on the surface of ER+ breast tumor cells forms heterotypic adherens junctions with N-cadherin in the osteogenic cells of the bone marrow and induces mTOR activation, a downstream effector of PI3K-Akt signal. Collectively, these bone metastatic traits arise with the oncogenic signaling and are intrinsically encoded in ER+ tumors.

**Gene Repurposing**

The second scenario, as exemplified in HER2+ tumors, is gene repurposing or gene re-exploitation. In primary tumors, Src facilitates ERBB2–ERBB3 heterodimerization and their kinase activation. Src is not essential for ERBB2-initiated oncogenesis, but having it provides additional survival advantage. This explanation agrees well with the observation that ~50% of HER2+ breast tumors are Src active. Amplifying the oncogenic signaling may not be critical for cancer cell growth in the primary tumor but may become critical and rate-limiting for survival when cells are threatened in the foreign bone marrow tissue. In similar ways, EREG, COX-2, MMP-1, and MMP-2 promote breast tumor growth via angiogenesis regeneration and vessel remodeling. These genes are reutilized for intravasation and extravasation purposes during metastasis. Genes normally involved in developmental programs or stemness maintenance may also be hijacked or reexploited for metastatic purposes. Indeed, if tumorigenesis is viewed as a process diverging from normal developmental regulation, cancer characteristics, including metastatic traits, are essentially a negotiation between the tumor-causing genes and the remaining normal genes. Melanomas are highly metastatic partly because of their neural crest origin, which is highly migratory in nature. On top of the oncogenic drivers, they may need few extra aberrations to metastasize. EMT transcription factors, such as Slug and Sox9, maintain mammary stem cell states in normal contexts and mediate long-term mammary gland reconstitution in mice. These genes in breast cancer are repurposed for mesenchymal and invasive phenotypes and contribute to the metastasis seedling of cancer cells. A similar finding points at another EMT controller ZEB1 as a determinant of the so-called cancer-stem-cell state. ELF5 and GATA3, normal regulators of...
mammary gland alveologenesis and LUM specification, are found to restrain EMT and suppress metastasis of breast cancer. Inhibitors of differentiation such as ID1 and ID3, genes thought to lock cell state, confer tumor reinitiation capabilities and sustain cancer proliferation during early metastatic colonization. This occurs partly through mesenchymal-to-epithelial transition (MET).\textsuperscript{96,97} Thus, these normal genes may be partly active or repressed during tumorigenesis, and their remnant activities may serve as metastatic traits.

**Stroma**
The third principle is the contribution from tumor-associated stroma and the microenvironment they shape. Querying features associated with Src activity enrichment that may explain the bone metastatic ability in the TN primary tumors failed to identify any biochemical links.\textsuperscript{89} This is in part consistent with the notion that TN breast cancer is a heterogeneous disease. Instead, the common feature shared by these Src-positive tumors is an enriched mesenchymal stroma. The mesenchymal stroma in many respects, including the cytokine composition, resembles the mesenchymal niches in the bone marrow. Such a primary tumor microenvironment nurtures the expansion of cancer cell clones with high Src activity and skews the carcinoma population toward a preponderance of clones with a predisposition to grow in the bone marrow. As a result, the mesenchymal stroma serves as a driving force for the bone metastatic trait in TN breast tumors. Thus, similarities between the microenvironment of primary tumors and that of the distant sites make the preselected metastatic seeds compatible with the target tissue and prime the development of organ-specific metastatic traits in primary tumors.

In addition to this example, it has been widely shown that different stromal components in primary tumors play critical roles in promoting tumor growth, increasing their invasiveness, and inducing the EMT phenotype.\textsuperscript{98} Fibroblasts, macrophages, and myeloid progenitors also secrete molecular cues that maintain the stemness of the cancer cells at the invasive front.\textsuperscript{99} Certain stromal cells promote metastatic colonization in an organ-specific manner in distinction to those in the primary tumors.\textsuperscript{64,100} Systemic responses elicited by the primary tumors may also cause behavioral changes in stromal cells, which in turn affect metastatic spread.\textsuperscript{101–104} Indeed, stroma is a co-evolving feature with tumor evolution and has many implications on the metastatic properties of tumor cells.\textsuperscript{42}

In summary, metastatic traits may arise from a combination of oncogenic forces, repurposing of intrinsic cellular properties, and stromal influences. An important lesson learned from the above findings is the relation of microenvironment similarity to the metastatic organ tropism. The distinction of distant sites from the primary sites has been long recognized and advocated.\textsuperscript{23,36} Even the best soil is still deadly overall for the disseminated cancer cells. Thus, what becomes critical is their chance to find a similar feature like their home from the vast sea of differences in the foreign tissue. Such a view is in line with Paget’s seed-and-soil theory\textsuperscript{24} and may help reveal new opportunities for therapeutic intervention against metastasis.

**Targeting Metastasis**
The identification of metastasis gene mediators provides new potential targets for treating metastatic breast cancer. The promise of these discoveries for translational medicine will be clinically evaluated in the future. Here, we discuss some of the foreseeable conceptual challenges of pushing these therapies into the clinic.

**Targets for Therapy**
Often times, when a patient is diagnosed with breast cancer, the primary tumor will be surgically removed and the residual disease will be eradicated by hormone, chemo, or radiation therapies. For patients who are at risk for developing metastasis, dissemination from primary tumors occurred prior to diagnosis and will cease with the radical surgery. Thus, it is futile to target the metastasis steps that already happened, including intravasation, circulation, and extravasation. These processes will be more meaningful targets for therapy within the context of multiple established metastases. Via preventing metastatic lesions from cross-seeding, self-seeding, or re-seeding to a tertiary site,\textsuperscript{105,106} these lesions can be locally confined and cytotoxic therapies can be more effectively applied one at a time. Owing to the transient nature of these processes, it may also be challenging to predict their occurrence and to develop corresponding therapeutic strategies. Thus, the clinical value of identifying these targets remains to be demonstrated.\textsuperscript{21} A more reasonable and practical target for clinical intervention is cancer cell survival or proliferation. The current systemic therapy and its extension to the adjuvant setting are designed exactly for this purpose. Whether it is hormonal (anti-ER) therapy against ER\textsuperscript{+} breast tumors, anti-HER2 therapy against HER2\textsuperscript{+} tumors, or chemotherapy that inhibits cell replication nonspecifically, all aim to sweep out proliferating cancer cells that remain in the patient’s body.

**Lessons from Hormonal Therapy and Anti-HER2 Therapy**
Despite recognition of the distinctions between metastases and their primary tumors, metastases are treated mainly based on their tumors of origin. This is based on the idea that metastases overall behave like their tissue of origin and many of the primary tumor characteristics, such as growth dependency and drug sensitivity, are maintained in metastases.\textsuperscript{20} Such a notion is supported by the success of hormonal therapy and anti-HER2 therapy for the two breast tumor subtypes, respectively. Indeed, any type of cancer treatment essentially aims to target a sensitivity window, within which cancer cells are selectively killed but normal tissues are spared.\textsuperscript{107} Thus, the wider this window is, the higher the chance tumors will be effectively eliminated. Based on such reasoning, targeting the oncogenic signaling (in the case of anti-HER2) or the
lineage peculiarity of growth demand (in the case of anti-ER) is an optimal treatment strategy. In line with this idea, many emerging targeted agents against other oncogenic signaling or pathways that were underappreciated in breast tumorigenesis are now being tested in multiple clinical trials. These include inhibitors against the PI3K-Akt–mTOR pathway, MAPK pathway, and JAK–STAT signaling; against RTKs such as IGF1R, FGFR, EGFR, ERBB3, and MET; against integrin signaling; against cell cycle regulators such as cyclin-dependent kinases (CDKs); and against epigenetic modifiers such as histone deacetylase (HDAC), DNA methyltransferase (DNMT), and bromodomain-and-extra-terminal (BET) domain chromatin modulators (summarized in Fig. 2). Not all these inhibitors are necessarily based on rationales and insights from metastasis biology, but in one setting or another, they are proven to have the ability to inhibit breast tumor growth. However, it is also foreseeable that without considering the contextual specificity of distant organ environments for therapy, relapse and resistance may occur in many cases. Drug resistance is a complicated matter of cellular intrinsic proliferation. The multilevels of interplay between cancer cells and stromal cells may render cancer cells such flexibility that when one proliferation signal is inhibited, another pathway can act on the cells and compensate. This highlights the complexity of established metastases.

Figure 2. A schematic shows key pathways of breast tumorigenesis and their targeted agents. Notes: Small molecules are highlighted in light shade; antagonist antibodies are highlighted in dark shade; * indicates multitargeted inhibitors. Abbreviations: RTK, receptor tyrosine kinase; AI, aromatase inhibitor; SERM, selective estrogen receptor modulator.
Intratumor Heterogeneity

The metastasis cascade is depicted as a delicate process that requires an intricate, coordinated action of multiple gene programs. If this is true, one may expect that it should be easy to disrupt the cascade and inhibit metastasis by abolishing any gene mediator of the process. This view is largely true on a single-cell level. However, metastasis occurs at the level of cell populations. Intratumor heterogeneity that is intrinsic to most of the metastases renders the metastatic traits versatile, redundant and complicated for targeting.

Analysis of single clones from the metastatic subpopulations toward a certain organ suggests that different clones within the aggressive subpopulation do not necessarily upregulate every metastatic gene, but rather harbor different subsets. This observation suggests that there is sufficient flexibility for different gene combinations to reach similar levels of metastatic fitness. In analogy to the intertumor heterogeneity seen in breast tumorigenesis, there exist multiple evolutionary routes for the breast tumor to reach the metastatic phenotype. As a result, inhibiting one mediator may eliminate part of metastasis but cannot eradicate it entirely.

Denosumab, an RANKL antibody, is in clinical trials to revert the osteolytic cycle of breast cancer bone metastasis and to inhibit bone resorption. However, as different metastatic clones can foster the vicious cycle in multiple alternative ways, the whole population develops resistance to RANKL inhibition and a cure is rarely achieved.

Moreover, the same genes and pathways may be repeatedly utilized for different purposes, functioning in a pleiotropic, context-dependent manner. The PI3K–Akt pathway is critical for cell survival in breast cancer metastasis to both bone and lung. However, the upstream signaling to reach this activation is quite distinct, with bone involving CXCL12 and IGF1 as cytokine triggers and with lung involving VCAM-1-mediated interaction with macrophages. VCAM-1 is important for both bone and lung metastasis from breast cancer.

Combination Therapy and Precision Medicine

Because of the intratumor complexity and the plasticity of metastases, it is expected that many of the targeted agents (Fig. 2) will show limited efficacies when applied singly and do not outperform the current standard-of-care in clinical trials. An emerging concept to overcome these issues is combination therapy, which combines multiple drugs and aims to inhibit multiple molecular targets simultaneously. Because effective first-line treatments exist for ER+ and HER2+ breast tumors, the primary focus of combination therapy in these two subtypes is to overcome acquired resistance and to achieve enduring treatment efficacies from anti-ER and anti-HER2 therapies. Resistance to hormonal therapy can occur through multiple mechanisms, including (1) reactivation of the ER pathway through ER mutation, ER protein modifications, or modulation of the ER genome-wide binding, (2) upregulation of RTKs, such as EGFR, ERBB2, IGF1R, and FGFR, and activation of the PI3K survival signaling, or (3) activation of CDKs and cell proliferation via other upstream pathways. Resistance to anti-HER2 therapy typically leads to reactivation of the PI3K signaling, either through (1) upregulation of the ERBB2 partners EGFR/ERBB3, (2) loss of PTEN, or (3) mutation of the PI3K pathway components. Thus, the inhibitors of these molecular targets are promising candidates to be included in the combination therapy (Fig. 2).

TN cancer, in contrast, presents a more substantial challenge. There is no targeted therapy available, and cytotoxic chemotherapy is the mainstay for TN tumor treatment. A special case where targeted therapy is available is the tumors with BRCA mutations, which show deficiency in one arm of the DNA repair pathways. BRCA-mutant tumors show extraordinary sensitivity to poly (ADP-ribose) polymerase (PARP) inhibitors and platinum chemotherapy, and can be treated using PARP inhibitors as single agents or in combination with chemotherapy. However, such therapy is not applicable to the majority of TN tumors, which are BRCA proficient. This view may be altered by an interesting finding showing that PI3K inhibition impairs BRCA expression and sensitizes BRCA wild-type tumors to PARP inhibitors in experimental models. This is further validated in some patients in a recent clinical trial of buparlisib and olaparib combination. Together, these findings demonstrate the potential of combination therapy and revive the hope of many targeted agents that previously were shown to be futile as single agents.

Another layer of combination therapy is to target the metastasis cascade mediators and block cancer–stromal cross-talk. Bisphosphonate and denosumab, inhibitors that block the osteolytic cycle, are well-known examples of drugs that can be combined with hormonal therapy for the treatment of ER+ bone metastases. Additional emerging agents of this category include inhibitors of WNT, NOTCH, and CXCR4 signaling (Fig. 2), angiogenesis inhibitors, and inhibitors of the immune checkpoint and immune modulators. PI3K–Akt pathway inhibitors have gained increased interest for the TN subtype, partly because many of the cancer–stromal interactions converge on the activation of this pathway and it controls metastatic survival as a central hub. Despite the many ongoing clinical trials, these inhibitors have proven only marginally effective as single agents. Therefore, what therapies these inhibitors should be combined with so that their potency could be leveraged becomes a major question for future research.

A fundamental challenge for combination therapy is the side effects that each drug carries. As the treatment potency...
increases with the drug combination, the toxic effects to the host tissue also worsen. Thus, the compound effects of the drug toxicities significantly limit the numbers of drugs that can be combined as well as the dose that can be applied for each drug. The success of combination therapy relies on a high therapeutic index and requires careful design of the combination regimen. As a result, to develop combination therapy with optimal efficacy, the therapy may have to be tailored from patient to patient, based on a more detailed understanding and more comprehensive characterizations of the metastasis lesions for each patient. With the advent of personal genomics and single-cell sequencing technologies and their wider application with patient biopsies in the clinic, precision medicine is no longer a farfetched idea and may be feasible in the future.\textsuperscript{128}

**Latent Metastasis**

Because of the many existing challenges in targeting established metastases, adjuvant therapy is designed to prevent metastasis formation, where the treatment is given after surgery and before any signs of disease recurrence. This protracted temporal gap between the primary tumor diagnosis and the emergence of metastatic foci in distant organs is referred to as metastatic latency or metastatic dormancy (Fig. 1).\textsuperscript{129} Adjuvant therapy is given at this time interval in an attempt to eradicate any microscopic lesions that escape detection and to minimize the risk of metastatic growth. As a result, latency is a temporal interplay between tumor progression and clinical detection/intervention.

At the cellular level, clinical latency may show as either solitary cancer cells or microlesions that are below the detection threshold in the clinic. These micrometastases may adopt a cellular state either entering quiescence or abortive growth with cell proliferation counterbalanced by cell death.\textsuperscript{130} Until now, most latent metastases in the clinic were detected in the form of DTCs in the bone marrow, because biopsies from other organs were difficult to obtain. Occasional reports of transmitting cancer to immunosuppressed recipients by organ transplantation highlight that organs beyond the bone marrow can harbor latent metastases as sanctuary sites.\textsuperscript{131} Detection of bone marrow DTCs in breast cancer patients has been shown to correlate with worsening patient prognosis and metastatic relapse at distant organs.\textsuperscript{132,133} However, the over prevalence of DTCs detected across tumor development stages\textsuperscript{134,135} raises the question of whether they remain relevant in the occurrence of metastasis or are merely a sign of tumor dissemination.\textsuperscript{23}

Despite the clinical importance of the dormancy state, few experimental models exist, leaving its biology unknown. It is believed that metastatic latency results from delayed adaptation of disseminated cancer cells to the foreign microenvironment.\textsuperscript{129} Such inadequacy may be because of cell intrinsic deficiencies,\textsuperscript{136,137} lack of neoangiogenic support,\textsuperscript{138,139} immune surveillance,\textsuperscript{140,141} or therapeutic enforcement.\textsuperscript{142,143} Adjuvant therapies show remarkable effectiveness in controlling ER\textsuperscript{+} and HER2\textsuperscript{+} breast tumors within the first five years, but a persistent risk of tumor recurrence exists beyond five years.\textsuperscript{10} This is explained by the effectiveness of the existing adjuvant therapy in targeting proliferating cells,\textsuperscript{144} but its inability to eliminate the latent cells that are nonproliferating or slow cycling. Though this notion requires definitive proof, it prompts deeper thought about the targeting of latent tumor cells for improved metastasis prevention.\textsuperscript{145-147} The major challenge now is how to merge the current findings with the well-established adjuvant regimens.\textsuperscript{148}

Even though the detailed knowledge about latent metastasis is still lacking, some basic nature of these cells can be inferred based on the current clinical and experimental evidences. First, there exist robust cell survival mechanisms that can support these cells during the latent period, even up to decades. The inability of most antimitotic therapies to eliminate metastases indicates that the machinery supporting latent cancer survival is different from that of cancer proliferation.\textsuperscript{149-151} Cancer cells at quiescence,\textsuperscript{152,153} slowly cycling,\textsuperscript{154,155} or displaying stem characteristics\textsuperscript{156} are distinct in drug sensitivity profiles compared to proliferating cells. Second, these latent cells maintain their proliferation potential and competence for cell cycling when the condition becomes congenial enough. Once they start proliferating, they may no longer depend on the latent survival mechanism. Thus, a drug effective against latent cancer cell survival may as well be futile against established, proliferating metastases. Such a possibility may let these targeted agents drop off the clinical trials easily, because most of these agents will be first tested in the advanced cancer settings in breast cancer clinical trials. Systematic therapies, including hormonal therapy, anti-HER2 therapy, and chemotherapy, are approved for the adjuvant setting because of their demonstrated efficacies in shrinking established tumors or metastases. Thus, the existing therapeutic regimens set a high standard for the latency-targeting agents.

Even if these inhibitors can be evaluated in the adjuvant trials, the readout of their clinical benefits remains obscure. Detection of CTCs, circulating tumor DNA, or circulating tumor transcripts remains not yet reliable enough for inferring disease progression, let alone predicting status of latent metastases.\textsuperscript{157} Repeated patient biopsy (for DTCs) is also too aggressive for merely assessing latent lesions. If the eventual occurrence of metastasis and patient survival serves as the end readout, patients may need to be monitored for more than 10–20 years. Such long-term investments, expensive efforts, and high-demanding support deter the enthusiasm of pharmaceutical companies. As a result, even though it seems quite appealing to target latent metastasis, many practical concerns impede such therapy from entering clinical testing. To overcome these limitations, a deeper understanding of the latency biology and convincing preclinical evidence of such therapeutic value are imperative.
Concluding Remarks

Metastasis remains the biggest hurdle for curing breast cancer. Recent findings have established a conceptual framework of cancer metastasis and provided deeper insights on the molecular basis of metastatic traits, their origins, and their evolution. How to incorporate this knowledge into the design of next-generation therapy is key to combating breast cancer metastasis. Different challenges exist for different breast cancer subtypes. TN breast cancers are the most aggressive and metastatic, with no effective targeted therapy available. Pursuing more potent and more specific therapies that reduce the first five-year recurrence rate is needed. For ER+ and HER2+ tumors, even though hormonal and anti-HER2 therapies are effective at controlling early diseases, a persistent risk of late relapse and drug resistance remains. How to combat drug resistance via developing novel combination therapy of chemotherapy, small molecule therapy, and immunotherapy is key to achieving durable therapeutic efficacies. Brain metastasis becomes an increasingly manifested challenge for HER2+ tumors. A deeper molecular insight of organ-specific metastases may guide novel therapeutic designs. To conclude, the past half century witnessed significant advancements in effective therapies for breast cancer, and we anticipate amazing breakthroughs in targeting breast cancer metastasis within the next 50 years.

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Author Contributions

Wrote the first draft of the manuscript: XJ. Contributed to the writing of the manuscript: XJ and PM. Agree with manuscript results and conclusions: XJ and PM. Made critical revisions and approved final version: XJ and PM. Both authors reviewed and approved of the final manuscript.

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