Breast Cancer Metastatic Dormancy and Relapse: An Enigma of Microenvironment(s)

Islam E. Elkholi[1,2], Andréane Lalonde[1,2], Morag Park[3], and Jean-François Côté[1,2,4,5]

ABSTRACT

Multiple factors act in concert to define the fate of disseminated tumor cells (DTC) to enter dormancy or develop overt metastases. Here, we review these factors in the context of three stages of the metastatic cascade that impact DTCs. First, cells can be programmed within the primary tumor microenvironment to promote or inhibit dissemination, and the primary tumor can condition a premetastatic niche. Then, cancer cells from the primary tumor spread through hematogenous and lymphatic routes, and the primary tumor sends cues systematically to regulate the fate of DTCs. Finally, DTCs home to their metastatic site, where they are influenced by various organ-specific aspects of the new microenvironment. We discuss these factors in the context of breast cancer, where about one-third of patients develop metastatic relapse. Finally, we discuss how the standard-of-care options for breast cancer might affect the fate of DTCs.

Breast Cancer: The Basics of the Disease(s)

Breast cancer is categorized into subtypes based on the expression of three receptors: estrogen (ER), progesterone (PR), and HER2. Gene expression–based studies have further categorized breast cancer into five subtypes: luminal A/B, HER2-positive, basal, and claudin-low (1–4). Luminal tumors are usually ER positive (ER^+), meanwhile basal and claudin-low tumors are ER negative. ER^+ tumors can also be HER2-negative ($HER2^-$) or positive (5). ER (hormonal)-negative and $HER2^-$ tumors (i.e., triple-negative breast cancer) are mainly, but not always (6), basal tumors. All of these subtypes can eventually develop metastatic relapse, accounting for an overall relapse incidence of approximately 25% of patients with breast cancer (7). However, differences in cumulative incidences, timing, and preference for sites exist (8–10). For instance, basal-like tumors tend to relapse within 2 years while luminal tumors can relapse up to 25 years later (11–13). Our incomplete understanding of the biological mechanisms of metastasis, and specifically the relapse phenomenon, underlies poor outcomes in patients (8).

[1]Montreal Clinical Research Institute (IRCM), Montreal, Québec, Canada. [2]Molecular Biology Programs, Université de Montréal, Montreal, Québec, Canada. [3]Rosalind and Morris Goodman Cancer Institute, McGill University, Montréal, Québec, Canada. [4]Department of Biochemistry and Molecular Medicine, Université de Montréal, Montréal, Québec, Canada. [5]Department of Anatomy and Cell Biology, McGill University, Montréal, Québec, Canada.

Corresponding Authors: Jean-François Côté, Montreal Clinical Research Institute (IRCM), 110 Avenue des Pins Ouest, Montréal H2W 1R7, Québec, Canada. Phone: 514-987-5647; E-mail: jean-francois.cote@ircm.qc.ca; and Islam E. Elkholi, Montreal Clinical Research Institute (IRCM), 110 Avenue des Pins Ouest, Montréal (QC) Canada, H2W 1R7. Phone: 514-987-5656; E-mail: islam.elkholi@umontreal.ca

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The Biology of Breast Cancer Metastasis: Where Does Dormancy Fit?

Metastasis: the inefficient silent killer

Breast cancer cells escape the primary tumor to reach the blood stream and become circulating tumor cells (CTC). CTCs then home to secondary organs as disseminated tumor cells (DTC) to eventually form macrometastases, hence the classical view that metastasis is a linear multistep cascade (**Fig. 1**), with selection pressure at every step. It has been estimated that 0.0004%–0.02% of primary tumor cells can be detected in the blood stream per day (14). Intriguingly, patients with large renal tumors shedding around 5×10^9 cells in the bloodstream per day were free of detectable metastases 5 years after surgery (14). The classic metastasis studies of Fidler provided relevant experimental insights into this phenomenon (15, 16). One hour after injecting B16 melanoma cells intravenously, about 50% of the cells were found in the lungs (15). Only 0.85% and subsequently 0.2% of the injected cells persisted in the lungs 1 and 14 days postinjection, respectively. Similarly, 80% of the cells injected through the intraportal route extravasated successfully by the third day and 1 in 40 cells formed micrometastases with 1 in 100 progressing to macrometastases (17). Intriguingly, 36% of the cells persisted as dormant solitary cells 13 days after injection. In breast cancer, the study of D2A1 and D2OR murine cells with high and low metastatic potential, respectively, provided insights into this process (18). Spontaneous and experimental metastasis assays using D2OR cells detected solitary cells in the liver, that did not undergo cell division for 11 weeks (18, 19). Even in mice harboring D2A1-overt metastases, dormant solitary cells were detectable. Collectively, these data suggest that inducing metastatic dormancy might be an inevitable fate and that reactivation from dormancy and progression from micrometastases to macrometastases are the main rate-limiting steps behind metastatic inefficiency.

Breast cancer metastatic dormancy: an outline

Although the concept of dormancy is relatively old, it remains poorly understood (**Fig. 2**). In 1954, Hadfield postulated that tumors that recur many years after excision at the metastatic site must have DTCs in a temporary mitotic arrest and he coined the term "dormant cancer cell" (20). The idea of having occult tumors at any point of our lifespan, or dormant metastatic lesions, is well founded and dates to the 1930s (**Fig. 2**; refs. 21–23). Two independent lines of evidence

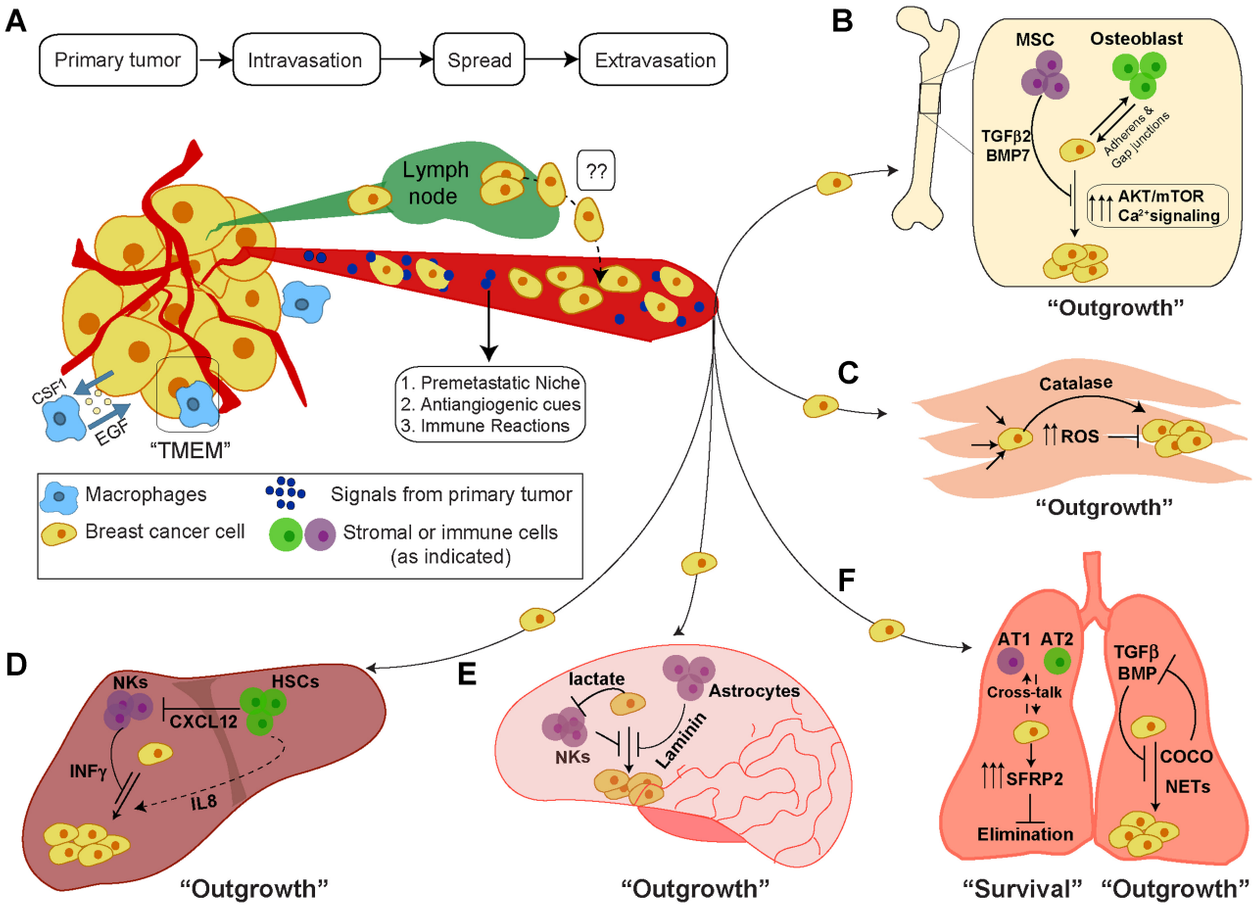

Figure 1.

Overview of the metastatic process and mechanisms regulating breast cancer dormancy. **A,** Schematic illustration summarizing the stations the cancer cells pass through before homing the secondary site. Different mechanisms can either induce and maintain the DTCs' dormancy, mediate their survival during dormancy, or induce their outgrowth. **B,** In the bones, osteogenic niche induces the outgrowth, meanwhile, NG2+/Nestin+ MSCs inhibit it. **C,** In muscle, high oxidative stress impedes metastatic outgrowth, and overexpressing catalase counteracts this effect. **D,** In the liver, NK cells induce the DTCs' quiescence, meanwhile, activated HSCs can alleviate this effect. *In vitro* work suggested that HSC-derived IL8 induces the DTCs' proliferation. **E,** In the brain parenchyma, astrocytes and NK cells can keep the DTCs in dormancy. DTC-secreted lactate counteracts the NK effect. **F,** In the lungs, DTC-secreted COCO and inflammation-induced NETs enhance the DTCs' outgrowth. Cross-talk between the lung cells (AT1/2) with DTCs upregulates SFRP2 in DTCs as a survival cue.

supported dormancy as an underlying biological mechanism behind metastatic relapse. One is the presence of DTCs in the bone marrow of patients with breast cancer at the time of primary tumor resection (24, 25). The second came from reports of organ transplant recipients who developed metastatic melanomas that could be traced back to the donors, revealing that the transplanted organs harbored dormant DTCs (26, 27).

DTCs can be found in two states: (i) cellular dormancy (19, 20) where the cells enter mitotic arrest and are in a solitary state; (ii) micrometastatic dormancy where cellular proliferation and apoptosis are at equilibrium in the metastatic tumor. Support for this latter state came from experiments showing that despite being mitotically active, the tumor mass could not progress due to failure in angiogenesis (28, 29), and that some micrometastases failed to progress to macrometastases (17). Immune-related mechanisms can also keep the tumor mass in "equilibrium" where proliferative cells are detectable but are unable to progress into larger lesions in the primary and metastatic settings (30, 31). Thus, micrometastatic dormancy can be further categorized into angiogenic and immunologic dormancy. Cellular and micrometastatic dormancy may collectively account for the clinical latency and the consequent relapses, specially that the identity of the metastasis-founder cells is still debated (13, 32). Whether metastases arise from solitary cells or clusters, or from early or late DTCs, considering the nonlinear parallel model of metastasis (33, 34), is not completely understood. Future studies investigating the dormancy-colonization switch dynamics in early and late DTCs might guide future clinically relevant definitions of dormancy (reviewed in refs. 13, 35).

Breast Cancer Metastatic Dormancy: Models, Current Landscape, and Challenges

Cell lines with unique *in vivo* metastatic behaviors

Currently, two breast cancer cell line pairs of different origins represent the main models to study dormancy. The first is the 4T1 and 4T07 pair of subclones originating from the 410 line, generated

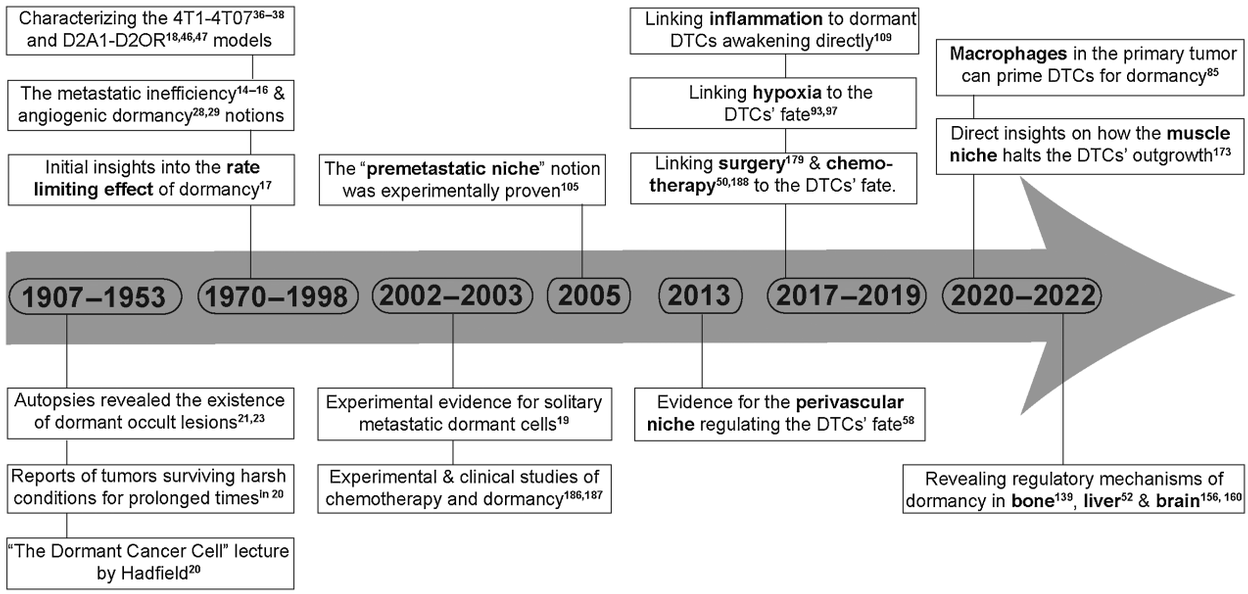

Figure 2.

Historical timeline for the landmark concepts and studies of breast cancer metastatic dormancy. Early studies complied evidence that cancer cells can form undetectable tumors in different settings, hence introducing the dormancy notion. The era of 1970s to the 2000s, witnessed establishing the metastatic inefficiency and angiogenic dormancy concepts. In parallel, key research models were generated and characterized. The following era (2000–2010) witnessed key insights about cellular dormancy (94, 95, 218) and metastasis in general such as the premetastatic niche. The last decade witnessed the integration between the tumor microenvironment and dormancy directions, with a clear exponential increase in studies revealing novel mechanisms involved in the dormancy dynamics (summarized in **Fig. 1**).

from a spontaneous metastatic nodule in the lungs of BALB/c mice bearing a breast tumor (36, 37). These two cell lines generate primary tumors once injected in the mammary fat pad of syngeneic BALB/c mice and can further disseminate to the lungs. The 4T1 cells form lung macrometastases while the 4T07 do not (38, 39). The 4T1-4T07 pair, as well as the 168FARN and 67NR that share the 410 origin but demonstrate lower metastatic potential, have been exploited to identify regulators of metastasis (40–42) and the dormancy-colonization switch (39, 43, 44). The D2A1 and D2OR cell lines originated from a D2 hyperplastic alveolar nodule (D2-HAN), a "preneoplastic" growth that originated spontaneously under the effect of high level of hormonal stimulation via pituitary isografts in BALB/c mice (45–47). These D2 lesions gave rise to mammary tumors upon inoculation in the fat pads, hence recapitulating the transformation process. After inoculation in the mammary fat pad, D2A1 cells generated lung macrometastases (in 4 weeks), whereas the D2OR failed (up to 13 weeks), despite being detectable in secondary organs (Supplementary Fig. S1A; refs. 18, 19), making this pair attractive for the study of the molecular and cellular basis of metastasis and dormancy (19, 48, 49).

Human breast cancer cell lines, such as the luminal ER^+ MCF7 (50) and T47D cell lines (51), have been used to identify regulators of metastatic latency (51). Other studies have exploited ER-negative and $HER2^-$ cell lines such as the highly metastatic MDA-MB-231 (52, 53) and its derivatives that display tropism toward different metastatic sites (54–56). For example, an MDA-MB-231 derivative (SCP6) demonstrated a 4-month latency before generating bone macrometastases (57). Cells isolated from the metastatic lesion were developed into cell lines that either escape dormancy such as the postdormancy generation 1 (PD1), PD2A/B/C/D/E or maintain dormancy such as PD2R (57). These studies and others provided significant insight into the dynamics of dormancy and colonization (58, 59).

Three-dimensional *in vitro* culture systems

Three-dimensional (3D) culture systems have been developed to recapitulate the microenvironments where DTCs reside (Supplementary Fig. S1B; refs. 48, 49, 60–62). One example is seeding cells on purified extracellular matrix (ECM) that mimics basement membrane (60–62). In this system, D2OR cells retain their quiescence and do not proliferate, while D2A1 cells are initially quiescent and then resume proliferating, hence mirroring their *in vivo* behavior. Similarly, a "Matrigel-on-Top" method, where cells were seeded on a layer of Matrigel, successfully recapitulated the *in vivo* behavior of D2OR and D2A1 cells. More recently, the D2OR/D2A1 pair was cocultured with lung cells and fibroblasts (48). To mimic the bone marrow microenvironment, a 3D-collagen biomatrix was developed in which cells of different breast cancer subtypes were cocultured with either permissive stromal cell–based or inhibitory endothelial cell–based and osteoblast-based niches (63). Wheeler and colleagues developed an *ex vivo* hepatic microphysiologic system in which breast cancer cells were cocultured with hepatocytes and nonparenchymal liver cells (64, 65). In parallel, organotypic microvascular niches were developed to seed cancer with stromal cells on a 3D microvascular network (58). These different systems revealed novel insights into DTCs' behavior and determinants of the dormancy-colonization switch.

Transgenic models

Transgenic breast cancer mouse models have been instrumental in understanding different steps of tumorigenesis, including metastatic latency. For example, the MMTV-Neu model that induces the

expression of activated HER2/Neu in the mammary glands (66) displays 100% incidence of primary tumors with about half of the mice progressing to metastases within 5 months (67–69). Similarly, the MMTV-PyMT model, that induces expression of the polyomavirus middle T-antigen in the mammary glands, generates primary tumors and metastases. Both models have been used to investigate dissemination onset. For example, it was demonstrated that DTCs can be found in the lungs and bone marrow of mice when lesions were only at the atypical ductal hyperplasia (ADH) or ductal carcinoma *in situ* (DCIS) stage (Supplementary Fig. S1C; ref. 33). Importantly, the question of whether these early DTCs are metastasis-founder cells was investigated. DTC-harboring bone marrow transplants from mice with ADH can develop macrometastases in wild-type mice challenged by irradiation, suggesting that the early DTCs can indeed bypass their initial growth arrest, and consequently initiate metastatic relapse. Other studies (70–72) have used these and similar models to reveal important features of the dormancy state over the last decade.

Current landscape and challenges

Challenges in the field of dormancy range from the scarcity of models that accurately recapitulate dormant phenotypes to technical and biological limitations of the available models. One example is the immunogenicity of fluorescent markers that can affect cell proliferation in a syngeneic background (73). Another is the authenticity of the 3D culture methods in recapitulating the *in vivo* microenvironment. In particular, the PI3K/mTOR inhibitor Gedatolisib failed to halt metastases *in vivo* despite its success in sensitizing quiescent breast cancer cells to doxorubicin in a bone marrow–mimicking organotypic culture system (74). These challenges have slowed down progress in understanding how the dormancy-colonization switch is regulated. What has become clear, however, is that fate of DTCs is determined at several stages before they colonize secondary sites: (i) at the primary tumor stage, (ii) in between primary and secondary sites (**Fig. 1A**), and (iii) in the secondary site (**Fig. 1B–F**).

Station 1: The Primary Tumor Influence on Metastatic Relapse

Priming the DTCs within the primary tumor microenvironment

The primary tumor microenvironment (pTME) includes different populations of tumor cells residing with various types of resident supporting cells as well as immune cells in a matrix of ECM nourished by tumor-initiated blood vessels (**Fig. 1A**). These components combine with the local biochemical environment (i.e., oxygen availability, etc.) to determine tumor aggressiveness and the DTCs' fate.

(i) Cellular component of primary TME. Despite the progress made in understanding the pTME's role in metastasis (75–77) and clinical outcomes (78), little is known about the direct correlation between the pTME and dormancy (79). The pTME residing cell type most linked to a role in dormancy is the macrophage that generally impacts breast cancer progression and clinical outcome (77, 80–82). In the PyMT model, macrophages accumulate in the primary tumor's perivascular regions to facilitate tumor cell migration and intravasation. Mechanistically, this depends on a paracrine signaling loop mediated by the tumor cell–secreted CSF1, to recruit and stimulate macrophages, and macrophage-produced EGF to mediate chemotaxis of tumor cells (83, 84). The resulting microanatomic structure has been named the "tumor microenvironment of metastasis" (TMEM; **Fig. 1A**). The TMEM is composed of the triad of a tumor cell, a macrophage, and an endothelial cell that together function as a doorway for intravasation, potentially facilitating early dissemination of breast tumor cells and further priming them for dormancy (85, 86). Tumor cells located near TMEMs, or cocultured with macrophages, exhibit upregulated NR2F1, a nuclear receptor acting as a pro-dormancy transcriptional regulator (87–89). Depleting macrophages reduced the number of NR2F1-positive lung DTCs. Whether quantifying TMEMs would be a useful prognostic tool for patients with breast cancer, given an earlier proof of principle (90), is under investigation. A TMEM-dependent automated score was found to be associated with distant relapse-free interval in patients with early (stage I–III) ER^+ $HER2^-$ breast cancer (91, 92). Intriguingly, however, this TMEM score negatively correlated with the Oncotype Dx score, the most advanced clinical test for predicting ER^+HER2^- breast cancer relapse. Therefore, the true clinical utility and validity of the TMEM score remains to be determined through large-scale clinical studies.

(ii) Biochemical and signaling aspect of pTME. Hypoxic microenvironments enrich for DTCs with upregulated NR2F1 and DEC2 dormancy markers that can maintain the dormant state for extended periods (93), although it is unclear whether this reflects what happens in spontaneous metastasis because cells were subjected to hypoxia after seeding on a chorioallantoic membrane, a model often used to study dormancy (94, 95), then injected intravenously in mice. Given the possibility of breast cancer cells' early dissemination (33, 71, 72) and the existence of hypoxic regions in human breast tumors at different stages (96), it is important to consider the onset and impact that hypoxia could have in the course of the disease. Indeed, fate tracing models have shown that hypoxic regions can be found as early as the DCIS stage and become more prominent as the tumor progresses (97). MMTV-PyMT mice expressing hypoxia-responsive Cre and tdTomato-floxed GFP transgenes have allowed monitoring of hypoxia during tumor progression by assessing the percentage of red and green cell at different timepoints. Using a parallel approach, it was shown that that hypoxia-primed MDA-MB-231 or 4T1 cells were more enriched in the blood stream and the lungs as compared with their percentage at the primary tumor, after orthotopic injection. This suggests that hypoxia-primed cells can bypass the rate-limiting steps of metastasis more efficiently. It would be interesting to harness these tools to assess the ability of normoxic and hypoxic early DTCs to seed metastases in secondary sites and to test whether exposure to hypoxia maintain a longer metastatic dormancy. Insights from such experiments might also guide the *in silico* modeling of DTC's fate and behavior in the context of different biological inputs (98, 99).

Primary breast cancer: a systemic disease

Surgical removal of 4T07 tumors resulted in failure of generating 4T07 cell colonies upon digesting the lungs and culturing cells *in vitro* (38), possibly because the primary tumor sends signals to support the DTCs' survival in the lungs. Emerging evidence supports this notion: breast cancer cells that spontaneously metastasize have a greater ability to be retained within the lungs, extravasate and consequently survive as compared with cells injected intravenously (85). Moreover, injecting the cells in the tail veins of mice bearing a primary tumor boosted their retention level in the lungs, up to 37% in comparison with 8% for cells injected in naïve mice. Given Paget's seed and soil hypothesis, it is appreciated now that the "soil" can be pre-prepared before the arrival of DTCs (100), and hence the view that cancer is a systemic rather than a localized disease (101–103). Indeed, the primary tumor mass produces cues, such as tumor secreted factors and extracellular vesicles (100, 104), that can define the DTCs' fate in the secondary site.

Primary tumors boost secretion of fibronectin in the metastatic sites and mobilize locally bone marrow–derived cells (BMDC, VEGFR1+ progenitors) before DTCs are detectable. These VEGFR1+ cells program the premetastatic niche that mediates the DTCs' adherence and growth on reaching these sites (105). Interestingly, different tumor types prepare the premetastatic sites in a preferential manner. Notably, melanoma B16 tumors prepared the metastatic niche in kidneys and liver, meanwhile Lewis lung carcinoma tumors did not (105). In the context of breast cancer, MDA-MB-231 cells acted as instigators by boosting the growth of cells with low tumorigenic abilities, acting as responders, through secretion of osteopontin (104), that relocated hematopoietic bone marrow cells to the responders' sites and enhanced their growth. Interestingly, subcutaneously injected MDA-MB-231 cells boosted the outgrowth of intravenously injected MDA-MB-231 cells already homing to the lungs, thereby leading to higher metastatic burden. Although this suggested an increase in the switch from micrometastases to macrometastases, no evidence was demonstrated at the cellular dormancy level (104). Recently, the RNA-binding protein Lin28b that regulates miRNAs (106) was demonstrated to mediate the formation of macrometastases in the 4T07 model by promoting a permissive premetastatic niche (107). The 4T07-Lin28b tumors secreted exosomes that induced local cytokine secretion in the lungs, resulting in recruitment and activation of N2 neutrophils that form an immunosuppressive premetastatic niche for DTC colonization.

Transcriptomic profiling of the lung niche during different stages of breast tumorigenesis in the PyMT model revealed a mechanism where breast tumors, in the premetastatic setting, instruct the Th type two cells to secrete IL13 that stimulates the lung mesenchymal stromal cells to produce complement C3 (C3; ref. 108). The latter in turn recruits and activates neutrophils to form neutrophil extracellular traps (NET) that boost metastatic colonization. Although this study did not focus on dormant lesions and their switch to macrometastases, it is plausible that this mechanism acts directly on dormant DTCs as the NETs can awaken dormant lung DTCs (109). Importantly, C3 levels were higher in patients with breast cancer with metastases than healthy counterparts, in line with another report demonstrating that C3 levels in primary breast and lung tumors correlated with developing leptomeningeal metastases (110). Whether C3 can act directly on dormant DTCs in these different contexts would be interesting to further investigate.

The primary tumor can also induce immune responses to either eradicate DTCs (111) or maintain their dormancy (112). In primary tumor-bearing mice, no macrometastases developed after intravenous injection of EMT6 and 4T07 breast cancer cells in contrast to tumor-free naïve mice (111, 112). Intravenous injection of cells after resecting the primary tumor led to the same result (111). Anti-CD8 antibody-based depletion of T cells in the two models reversed this phenotype and led to macrometastases formation. Comparison of the 4T1- and 4T07-derived primary tumors revealed that the latter contained a higher frequency of $CD39^{+}PD\text{-}1^{+}CD8^{+}$ T cells (112). Transfer of the latter population to mice mimicked the effect of primary tumor presence on halting macrometastases after intravenous 4T07 cell injection. Furthermore, treating mice with anti-PD1 antibody diminished the number of residual dormant lung DTCs after tumor resection. These two studies suggest that: (i) primary tumors can suppress the metastatic colonization in an immune-mediated manner; (ii) breast cancer cell lines initiate differential immune responses including a differential representation of immune cells within the primary tumors as well as recruiting different populations at the secondary site; (iii) immune checkpoint blockade approaches (i.e., anti-PD1/PD-L1) might be of benefit for preventing metastatic relapse, as currently being tested in the PALAVY trial (NCT04841148).

Breast tumor–secreted enzymes, such as lysyl oxidase (LOX), can also impact the DTCs' fate. LOX, through collagen crosslinking, mediates the formation of a fibrotic lung microenvironment, a character associated with awakening of dormant DTCs (109, 113). Moreover, LOX secreted in higher quantities from breast cancer cells and/or tumors under hypoxia than normoxia is also required for the formation of bone metastases (114). In parallel, primary tumor-secreted LOX facilitates the recruitment of BMDCs to setup the premetastatic niche that facilitates lung colonization (115). LOX can also be secreted by cancer-associated fibroblasts (CAF) where it contributes to metastatic progression (116). Depletion of CAFs in the primary and metastatic sites and the consequent reduction in LOX levels is associated with a lower metastatic burden in the 4T1 model (117). Whether CAFs play direct roles in the dormancy-colonization switch through LOX would be interesting to dissect in future studies.

Altogether, these studies support the notion that breast cancer is a systemic disease and motivate the search for secreted signals. These signals can be categorized as: (i) cytokines and chemokines that recruit and instruct the immune cells or the stromal cells in the secondary sites; (ii) enzymes acting directly on the secondary TME. These signals can either promote or inhibit metastatic colonization.

Station 2: DTCs in Transit between Primary and Secondary Sites

Cancer cells spread through both the bloodstream and lymphatic system to reach secondary organs (**Fig. 1A**). In the bloodstream, they can overcome anoikis, immune surveillance, and mechanical shear forces to survive. Whether these events prime the CTCs, and sequentially the DTCs, for dormancy is poorly explored. However, some results from available literature provide interesting insight into this question. First, cancer cells can directly engage with platelets to be shielded from immune cells in the circulation, which ensures maintenance of an invasive trait that facilitates their extravasation and proliferation in secondary sites (118, 119). This is mediated mechanistically by platelet-derived TGFβ, and specific pathways in the CTCs including NFkB signaling (119, 120). Second, disseminating cells with the ability to resist anoikis (cell death induced by detachment from ECM) might be more efficient in generating metastases (121–123). Third, breast CTCs primed with hypoxia in the primary tumor are more resistant to oxidative stress in circulation (124) and demonstrate better survival (97). Whether the cancer cells carry a specific memory from the CTC stage that will affect their behavior/fate once landed in the new site remains to be explored. Recently, a genome wide gain-of-function CRISPR screen in patient-derived CTCs defined RPL15, a structural component of the large subunit of the ribosomal machinery, as a metastasis promoter (125). This study also suggested that maintaining an epithelial state in the CTCs might allow them to upregulate their translational machinery and proliferative power upon homing to the lungs. This indicates that if the disseminating cells are primed in the primary tumor or in the circulation, they might be able to revoke their initial dormancy and colonize. Two points to be noted in such a direction, the period that cancer cells spend in the circulation between the two tumor sites and the directionality of dissemination. Is homing to the secondary site a dead-end for cancer cells? Detecting CTCs in primary tumor-free and overt metastases-free patients with breast cancer up to 22 years postmastectomy argues against a dead-end notion (126). Furthermore, CTCs can reseed back to primary tumor

site (127) and bone-homing dormant DTCs can return back to the circulation (128). Whether these dynamics play a role in regulating the dormancy-colonization switch is not known and more research is warranted into the role of the dissemination process itself.

In contrast to the hematogenous spread, the lymphatic spread is less studied. Initially, it has been thought that reaching the draining lymph nodes is a dead-end for cancer cells (119). However, more recent work proved otherwise. Intralymphatic infusion of the 4T1 cells led to pulmonary metastases in the absence of primary tumor (129). Three days after infusion, the 4T1 cells infiltrated the node structure, and intravasated through its blood vessels. Interestingly, even after excising the lymph nodes 3 days after infusion, lung metastases were detectable in one-third of the mice 8 days later. In a back-to-back study, 4T1 cells harboring a photoconvertible fluorescent Dendra2 protein targeted to the nucleus were injected in the mammary fat pad and primary tumors were resected eventually (130). Local photoconversion on the lymph nodes allowed the conversion of the fluorescent protein from green to red and hence enabling tracing the cancer cells. Like in the Brown report (129), the red 4T1 cells were detectable in the blood stream and the lungs. These studies argue strongly against a "dead-end" notion by demonstrating that breast cancer cells can use the sentinel lymph nodes' blood vessels as an exit gate toward the blood stream (**Fig. 1A**). Whether cancer cells are primed in the lymph nodes to maintain or exit dormancy in the secondary organs is still unexplored.

Station 3: At the Metastatic Niche, the Final Stage on the Road

Upon reaching their new "home," DTCs will be faced with either hospitable and/or hostile conditions as they encounter the tissue's specialized ECM in addition to stromal and immune cells. For instance, it has been shown that collagen III enriched ECM can induce and sustain dormancy in different tumor models (131). Furthermore, DTCs' neighboring microvascular basement membrane can regulate the fate of DTCs (58). In particular, DTCs near the endothelial cells at the sprouting tips have been reported to proliferate while those that reside near the stable microvascular endothelium stayed quiescent through the effect of differential endothelial cell–derived factors. Thrombospondin-1 (TSP-1) is enriched where the DTCs remain quiescent, while TGFβ1 and periostin (POSTN) are enriched around the neovascular tips. Cues such as POSTN can also be secreted by stromal cells to support the stemness and metastasis-initiating potential of DTCs (132). Consistent with the angiogenic switch notion, latent nonangiogenic tumors (palpable after 119 days after inoculation) secrete higher levels of the angiogenic repressor TSP-1 than angiogenic tumors (palpable within 19 days; ref. 133). In addition, TSP-1–overexpressing primary breast tumors have a 50% decrease in the incidence of lung metastasis as compared with the unmodified tumors (134). These findings suggest that the same cues secreted or deposited by different sources can dictate DTC fate. DTCs can also engage with the immune system (31). Koebel and colleagues showed that the adaptive immune system can keep the tumor mass in an equilibrium, which can be anticipated for the micrometastatic or proliferative DTCs clusters (135). In the context of breast cancer metastasis, quiescent DTCs evade the natural killer (NK)-cell surveillance and clearance while maintaining a stem-like state that enables their colonization (136).

Together, these findings have highlighted the role of secondary site microenvironment-imposed regulatory mechanisms of the DTCs' fates: (i) death and clearance; (ii) inducing and maintaining dormancy; and (iii) metastatic outgrowth (**Fig. 1B–F**). We will focus on the latter two fates of DTCs in an organ-centered view given the niche specificity in such organs (see ref. 137 for details of such niches).

Bone marrow

A specific spatial organization for breast DTCs in the bones was reported (50, 128). Remarkably, dormant DTCs localized in the perisinusoidal niche while the proliferative DTCs localized in the nonsinusoidal regions. This pattern was further extended to patient samples, as indicated by the Ki67-staining status. This prompted the idea that neighboring cells might have a role in regulating the dormancy-colonization switch, as was demonstrated recently by different groups (**Fig. 1B**). The NG2+/Nestin+ mesenchymal stem cells (MSC) residing in the bone marrow can maintain breast cancer cells dormancy. Depleting these MSCs led to a 10-fold increase in the incidence of high metastatic burden (defined as > 1,000 breast cancer cells/1 million bone marrow cells) potentially via decreasing the levels of TGFβ2, a pro-dormancy cue that upregulates the p38 signaling pathway (138, 139). Depleting TGFβ2 specifically in the NG2+/Nestin+ MSCs mimicked depleting these MSCs but to a lower level. In addition, the levels of TGFβ2 and BMP7 were found to be more detectable and enriched in treatment-naïve patients with nonmetastatic early breast cancer as compared with those with overt metastases. However, patient sample size was rather small in this study and follow-up reports assessing these associations on a bigger scale should consider the patients' age given the possibility of the TGFβ2 and BMP7 levels might vary with age. In another study (140), 4T07 and MCF7 cells were found to reside in an osteogenic niche of the lower limb bones, composed mainly of cells positive for the osteoblast markers alkaline phosphatase and collagen I (141). These DTCs formed adherens junctions with the osteogenic cells, which induced the AKT/mTOR pathway in the DTCs and enhanced the metastatic burden in the bones (**Fig. 1B**). Targeting this pathway pharmacologically diminished the spontaneous metastatic burden in the bone but not the lungs, suggesting niche specificity. Further work showed that the connection between the DTCs and osteogenic cells facilitates Ca^{2+}-dependent signaling and the metastatic outgrowth (**Fig. 1B**). Interfering with this circuit pharmacologically and genetically halted the outgrowth of micrometastases in different models (142). Cross-talk between DTCs and osteoclasts also mediates bone metastasis formation through upregulation of VCAM1 to facilitate the colonization step (57). Depleting VCAM1 minimized the metastatic burden in mice injected with the MDA-MB-231 derivative PD2D cells, potentially due to reduced recruitment of osteoclast progenitor cells. Whether VCAM1 can also regulate dormancy on a cellular level would be interesting to explore further.

Lungs

3D and coculture systems yielded novel insights into the impact of the lung niche on DTC fate (48, 49, 60, 61). Alveolar type 1 (AT1)-like cells can support the survival of D2OR cells while inhibiting their proliferation induced by the AT2-like cells (48). The same study identified *Sfrp2* as a prosurvival gene for dormant lung DTCs. Interestingly, AT1 cells upregulated the expression of Sfrp2 in the dormant D2OR cells to boost the D2OR deposition of fibronectin and mediate the formation of cellular protrusions, hence stimulating different signaling pathways to mediate the DTCs' survival (**Fig. 1F**). Another study suggested that lung stroma–derived TGFβ/BMP inhibits 4T07 DTCs' colonization. COCO, a protein secreted by breast cancer cells, counteracts this signaling pathway to induce the metastatic outgrowth in the lungs but not in bone or brain (39). The genetic screen that identified COCO also predicted the long noncoding RNA (lncRNA)

Malat1 as a colonization signal (39, 43). However, a subsequent investigation suggested that *MALAT1* is a metastasis suppressor in breast cancer progression (143). Mice bearing *Malat1*-deficient primary tumors had higher numbers of CTCs and harbored more lung metastases than control animals. *MALAT1* was also found to be upregulated in dormancy-prone cells when compared with their fully metastatic counterparts (144). Whether *MALAT1* plays a role post-extravasation in the dormancy-colonization switch is unclear. *NR2F1-AS1* was also identified as a pro-dormancy lncRNA for breast cancer cells in the lungs. Liu and colleagues found that despite having an increased ability to invade and disseminate, mesenchymal-like breast cancer stem cell-like cells (mBCSC) are more prone to metastatic lung dormancy than epithelial-like BCSCs (144). Depleting *NR2F1-AS1* in these mBCSCs induced a mesenchymal–epithelial transition (MET) and reduced the number of lungs DTCs, but eventually enhanced the metastatic burden by increasing the proliferation of DTCs. Molecularly, *NR2F1-AS1* enhanced the translation of NR2F1, which in turn suppressed the expression of the MET-inducer miR-205. While these studies highlight the basal effect of unchallenged normal lung tissue on the fate of breast DTCs, other studies focused on pathologic states, such as lung inflammation, that are linked to breast cancer metastasis (145) and patient outcomes (146). Induction of lung inflammation recruited and activated neutrophils to form NETs that led to awakening of dormant DTCs (**Fig. 1F**). Mechanistically, the NET-associated proteases metalloproteinase 9 and neutrophil elastase remodeled the ECM by cleaving laminin and activating an integrin-dependent pathway in the D2OR cells to mediate their awakening (109). Interestingly, comparing the 4T1-4T07 pair indicated that the 4T1s are more capable of inducing NETs than the 4T07s (147). These NETs can subsequently boost the cells' invasiveness and eventually the lung metastatic burden. Collectively, these data demonstrate that inflammation and NET formation can dictate DTC fate. Interestingly, chronic inflammation can alter the repertoire of the AT1 and AT2 cells (148); whether the latter is a factor in awakening the dormant DTCs remains to be determined.

Liver

Although, progress has been made in identifying genes and phenomena associated with breast cancer metastasis to liver (40, 149–151), direct studies on the dormancy phase are still scarce. Chemokines, such as IL8 secreted by the hepatic stellate cells (HSC), and exosomes in the hepatic niche have been reported to either promote or halt the proliferation of breast cancer cells in two-dimensional coculture and a hepatic microphysiologic system (**Fig. 1D**; refs. 152, 153). A more in-depth study (52) reported that DTCs occupied different areas of the liver and existed in three different patterns: macrometastases, clusters (< 10 cells) or single quiescent cells. Transcriptomic analyses of the dormancy-associated stroma indicated an enrichment for genes related to NK cell–mediated responses. Indeed, NK cells' levels showed a descending gradient from the dormancy-associated stroma followed by stroma from tumor-free mice to the lowest level in the macrometastases-associated stroma. Depleting the NK cells in mice bearing dormant 4T07 DTCs increased the metastatic burden, meanwhile expanding them by IL15 treatments in mice bearing 4T1 or MDA-MB-231 decreased it. In addition, the chemokine CXCL12 secreted by activated HSCs in the hepatic niche decreased the proliferation of NK cells and contributed to metastatic outgrowth (**Fig. 1D**). Indeed, the levels of NK cells and activated HSCs have also been found to be inversely proportional in biopsies from liver-homing breast cancer metastases, although the breast cancer subtype of these patients was not indicated. It might be clinically relevant to determine whether luminal breast cancer cells homing to the liver undergo the same dynamics, given that approximately 30% of those patients relapse in the liver (8). Furthermore, estrogen receptor 1 (ESR 1)-activating mutations are enriched in ER^+ liver metastatic relapse (154).

Brain

Brain metastases occur in two different subanatomical structures; parenchymal and leptomeningeal and these niches exhibit different selective pressures on DTCs (110).

(i) Parenchyma. The brain microenvironment is hostile to metastatic formation and limits it at different stages (155–158). Brain parenchyma limits cancer cell invasion and survival through the protein plasmin, that DTCs counteract by secreting serpin proteins to initiate their colonization (155). The lncRNA *BMOR* has also been reported to promote breast cancer cell brain colonization, possibly through evasion of immune clearance (159). Two recent reports have addressed the role of dormancy in this process. Metabolic analysis of breast cancer cells with differential metastatic behavior in the brain demonstrated that latent metastatic cells secrete lower levels of lactate than those that successfully generate macrometastases (160). Administering lactate to mice bearing latent metastatic cells mediated formation of macrometastases, potentially by promoting escape from innate immune surveillance (**Fig. 1E**). A parallel study showed that the astrocytes in the perivascular niche deposit laminin that acts through DTC dystroglycan receptors to suppress yes-associated protein (YAP) activity in these cells, resulting in an inhibition of their proliferation (**Fig. 1E**; ref. 156). This study further demonstrated that parental breast cancer cell lines and their brain-tropic derivatives showed similar incidences for clearance and extravasation upon arrival in the brain but differed mainly in the switch from dormancy to micrometastases and macrometastases. However, this comparison was done in the context of experimental, not spontaneous, metastasis where differences in DTC fate have previously been reported (85). DTCs can also impose changes in the brain microenvironment (158, 161, 162). For example, brain metastatic breast cancer cells upregulate the *Lnc-BM* lncRNA, which induces a JAK2/STAT3 signaling axis that in return stimulates the secretion of CCL2 (163). This cytokine recruits brain macrophages to secrete additional cytokines (i.e., oncostatin M and IL6) to eventually stimulate JAK2 signaling in the breast cancer cells in a positive feedback loop. Although the elucidated mechanism serves mainly to enhance DTC extravasation and adhesion to brain endothelium, an effect on the dormancy-colonization switch is also possible. *In vivo* depletion of *Lnc-BM* 3 to 9 days after intracardiac injection of breast cancer cells [when most cells would have extravasated (156)] decreased the formation of macrometastases. Investigating whether *Lnc-BM* directly regulates dormancy would be of interest.

(ii) Leptomeninges. Leptomeninges refer to two of the three meninges surrounding the brain and spinal cord: pia matter and arachnoid and the cerebrospinal fluid (CSF)-filled subarachnoid space in between them. Cells capable of growing in growth factor–free CSF are predicated to modulate this microenvironment to their benefit, as supported by two reports (110, 164). Indeed, cancer cells upregulate the iron-binding protein lipocalin-2 (LCN2) and its receptor SLC22A17 allowing them to take up extracellular iron available in the CSF. Genetic depletion of LCN2 or SLC22A17 or administering iron chelation therapy minimized the incidence of leptomeningeal metastasis in mouse models. Despite these efforts, direct studies on dormancy in this anatomic structure are still lacking.

Skeletal muscle

Although clinically detectable muscle metastases from the breast is a rare event (165, 166), a few relapse cases have been reported in the literature (167–169). For instance, a patient with an ER^+ node-positive breast cancer was diagnosed with a metastatic relapse in her biceps muscle 25 years after the primary tumor incidence (170). Different hypotheses were proposed to explain this rare event, including biophysical conditions such as blood flow and temperature (170), biomechanical destruction of the muscle DTCs (171), high lactic acid levels in the muscles that intervenes with the metastasizing cells' attempts to induce angiogenesis and hence failure of establishment (172). However, direct and mechanistic experimental insights have been lacking until a recent study (173): Crist and colleagues first showed that MDA-MB-231 cells can spontaneously disseminate and home to muscles, suggesting that outgrowth in the muscle microenvironment might be the main rate-limiting step in developing metastases. High levels of oxidative stress (i.e., reactive oxygen species) in muscle-homing DTCs halt their colonization (**Fig. 1C**; ref. 173). Even in permissive microenvironments such as the lungs, early DTCs show high levels of oxidative stress that diminish with progression toward micrometastases and macrometastases. Consequently, cells expressing catalase, to overcome the oxidative stress, could colonize the muscles and form macrometastases (**Fig. 1C**). This study raises a plethora of questions and potential connections to new research directions. First, exercise is closely related to reactive oxygen species production and redox in the muscle niche (174, 175). How would regular exercise affect the DTCs' fate? Would a difference be noticed if mice were subjected to exercise before intramuscular implantation of the breast cancer cells? Second, a study documented that muscle metastatic relapse took place in a previous site of trauma in 8 patients, including 2 with breast cancer (176). Whether this is a recurrent phenomenon is not established and whether there is a mechanistic explanation for this type of metastatic outgrowth is not known. Following a trauma, a multistep regeneration program replaces damaged muscle fibers with healthy ones. Muscle stem cells proliferate, differentiate, and eventually fuse to form multinucleated muscle fibers (177). The proposal by Crist and colleagues that differentiated myoblasts can inhibit metastatic outgrowth raise the question of whether inducing a muscle injury before intramuscular implantation of breast cancer cells could change their fate. Crist and colleagues analyzed autopsy-obtained muscle samples, from the quadriceps, gastrocnemius, and tibialis anterior of 2 patients with ER^+ metastatic breast cancer and detected DTCs. Whether all breast cancer subtypes disseminate to the muscle niche and/or have an anatomic preference for the type of muscles to home to as well as their capacity to survive in these sites is unclear. Autopsy research might provide insights into these questions as it did for dormancy almost a century ago (21, 178).

Therapeutic Management of Dormant Metastases: Current Landscape

Regardless of the clinical management approach of patients with breast cancer (i.e., surgery, chemotherapy, radiotherapy, or targeted therapy), the fact that relapse happens suggests that the dormant DTCs can survive and evade these treatments. In this section, we will discuss relevant findings that outline the current understanding and goals in preventing metastatic relapse.

Surgery

Primary tumors emit antiangiogenic signals that inhibit angiogenesis at the secondary site, hence limiting the switch from micrometastates to macrometastases (29). Primary tumor resection relieved this inhibitory effect. Furthermore, treatments with antiangiogenics after the tumor resection kept the micrometastases at bay, suggesting that resection influences DTC fate and that antiangiogenics can prevent relapse. Krall and colleagues sought to separate the effects of tumor removal from the effects of the surgical wound and the healing process on the growth of distally located D2A1-GFP tumors (179). Strikingly, performing a "mock" surgery where sterile polyvinyl acetate sponges were implanted subcutaneously either before or after inoculating the D2A1-GFP cells bypassed the expected immune rejection (73), and led to a higher incidence and volume of tumors. Notably, treatments with anti-inflammatory agents after inducing a surgical wound, but before implanting the D2A1-GFP cells, decreased the tumor burden. This study justifies considering anti-inflammatory agents along with resecting primary breast cancer (179, 180). On the other hand, regardless of their early promise (181–183), antiangiogenics failed to achieve clinical success in breast cancer when used in different settings and combinations (184). Furthermore, antiangiogenics could induce the lung DTCs' outgrowth when administered before or after intravenous injection of breast cancer cells (185). Despite halting the primary tumor, the antiangiogenic treatment stimulated the lung DTCs' outgrowth after resecting the primary tumor. These findings argue against a clinical utility for antiangiogenics in breast cancer metastatic relapse, at least until a better understanding of the underlying biology is attained, as suggested before (184).

Chemotherapy

In a group of 59 patients with metastasis-free breast cancer, the effect of chemotherapy on the bone marrow DTCs, identified by cytokeratin staining (CK), was assessed (186). About 50% of patients were CK+ both before and after chemotherapy treatment. While 50% of the initially positive patients became negative after the treatment, 37% of the initially negative patients had detectable CK+ DTCs after chemotherapy. The existence of these posttreatment CK+ DTCs was also a prognostic factor for distant metastasis relapse and death. This chemoresistance of dormant DTCs has been attributed to their quiescent noncycling state (187). However, a recent study (50) challenged this view by showing that DTCs localized in the perivascular niche are resistant to chemotherapy via an integrin-based mechanism. Indeed, inhibiting different integrins sensitized the cancer cells to doxorubicin. This study further energized the search for druggable downstream effectors of integrin signaling (74). However, this approach did not achieve the goal and was more challenging than anticipated (74).

Chemotherapy can also enhance TMEM-dependent dissemination rates, leading to more micrometastases despite decreasing the primary tumor burden (188). Kreso and colleagues used lineage tracing and serial tumor transplantation in mice to demonstrate that chemotherapy could shift an initially dormant clone to a dominant clone in the tumor, despite being barely detectable before treatment (189). These studies and the observed consequences of chemotherapy on metastasis (190, 191) raise certain questions: In the Braun and colleagues report (186), did chemotherapy have a negative role in the patients where DTCs were only detected after treatment? Was there a selection pressure that allowed these DTCs to emerge?

Endocrine therapy

Metastatic relapse occurs in 13% to 41% of patients with ER^+ breast cancer (total $N = 63{,}000$) over 15 years after the standard 5-year endocrine therapy (ET), suggesting that dormant DTCs can evolve

under ET (192). Mechanistically, standard-of-care ET (i.e., tamoxifen and fulvestrant) selects for a $CD133^+$ stem-like cells that bypass an initial metabolic dormancy through upregulating IL6, and thereby regain their self-renewal ability to induce bone metastases (193). The significance of IL6 as a reactivation signal was also supported in an independent study using, among other investigations, single-cell transcriptomic analyses of breast cancer patient-derived bone marrow DTCs (194). However, a clear understanding of the ET's contribution to the metastatic microenvironment is lacking.

In addition to adjuvant ET, patients with ER^+ breast cancer might in some cases receive bone-modifying agents such as zoledronic acid (ZA). Despite the lack of mechanistic explanations, ZA was linked to a reduced bone marrow DTC burden (195) and a further decrease in incidence of bone metastatic relapse especially in postmenopausal women (196, 197). One possible explanation is that ZA manipulates the hematopoietic cells profile to suppress breast cancer cells growth (198). Further rigorous work is needed to understand the direct effect of ZA on the fate of bone DTCs.

Targeted therapy

Metastatic relapse can also occur in the context of targeted therapies such as trastuzumab, a monoclonal anti-HER2 antibody and standard of care for patients with $HER2^+$ breast cancer (199). Such relapses are associated with cancer cell–intrinsic adaptations at the genomic (199) and potentially metabolic levels. For instance, NRF2, an antioxidant transcription factor, was identified as a promoter of local recurrence following inhibition of HER2 (200). Although, mechanistic HER2-focused studies have been reported (71, 72, 201), more clinically oriented work focusing on HER2 targeting and the dormancy-colonization switch is needed. The receptor tyrosine kinase AXL has also been implicated in breast cancer progression and metastasis (68, 69), making it an attractive target in clinical settings. AXL is upregulated in bone dormant myeloma cells (202), and its overexpression reduces the number of Ki67-positive lung DTCs in melanoma (203). Intriguingly, this role is not universal between cancer lineages, as depleting AXL in $HER2^+$ DTCs after extravasation reduced metastatic burden (68). In $HER2^+$ breast cancer, AXL mediates its metastasis-promoting actions independently from its ligand GAS6, which was recently defined as an upregulated gene in dormant lung and bone DTCs (204). Although manipulating the expression level of GAS6 in breast cancer cells did not alter the metastatic burden *in vivo* (204), stromal GAS6 was needed for successful lung metastasis (205). These results underscore the need for a better understanding of the role of the GAS6-AXL circuit in breast cancer dormancy-colonization dynamics, especially with the availability of the specific AXL inhibitor Bemcentinib, that could be used to counteract this pathway.

Dormancy-focused therapeutic approaches

Autophagy has been proposed as a survival mechanism for dormant cells in different lineages (62, 206, 207). For example, inhibiting autophagy with hydroxychloroquine reduced the viability of D2A1 and D2OR breast cancer cells during dormancy. These findings have motivated the ongoing CLEVER (NCT03032406) and PALAVY (NCT04841148) trials that assess the efficacy of hydroxychloroquine individually or in different combinations in preventing relapses in patients with breast cancer who harbor DTCs in their bone marrow without evident metastatic lesions. However, two points call for caution. First, escaping from dormancy toward colonization was accompanied with reduced autophagy in another study (208). Perhaps the DTCs' burden (high vs. low), and the timing of the intervention (i.e., just after homing the lungs vs. later; ref. 62), might dictate the net effect of autophagy inhibition on the DTC fate. Investigating this will be crucial for clinical applications. The interconnection between mTOR complex 1 (mTORC1) activity and autophagy induction should also be considered (209–211). Although it is plausible that dormant DTCs depend mainly on autophagy for their survival under stressful conditions while suppressing mTORC1 activity, high mTORC1 activity is also a survival mechanism for the dormant DTCs (212). Hence, combining mTOR and autophagy inhibitors might be a better strategy (213) and this notion remains to be explored in breast cancer. Whether different microenvironments or breast cancer subtypes select for and/or have a preference between the two survival mechanisms is unclear at this point.

The dormancy-colonization switch is an example of behavioral plasticity in cancer (214) that includes reversible EMT/MET and the sequential acquisition of stemness-related characteristics (119, 214–217). Indeed, dormant DTCs resemble stem cells, particularly in their retention of self-renewal ability while being quiescent (39, 88, 144, 218). Interestingly, treatment with the FDA-approved DNA demethylating drug 5-azacytidine (AZA) and all-trans retinoic acid (atRA), in an NR2F1-dependent mechanism, induced expression of the pluripotency mediators NANOG and SOX2 along with growth inhibition in different lineages including breast cancer (88). This motivated an ongoing clinical trial for AZA and atRA to enforce dormancy and prevent relapses in patients with prostate cancer (NCT03572387). Whether such an approach can be successful in breast cancer is unclear, given the incomplete understanding for the role of NR2F1 in breast cancer plasticity. In one study, NR2F1 mediated a mesenchymal invasive phenotype (144), but in another, NR2F1 blocked dissemination in early $HER2^+$ breast cancer lesions, where its loss reduced E-cadherin and increased TWIST1 levels (201). These results suggest context-dependent NR2F1 roles and regulation between not only different BC models but also at different timepoints (i.e., early vs. late) in breast cancer lesions (201).

Conclusions and Future Perspective

The decision of whether to enter, maintain, or escape dormancy at the secondary site is clearly affected by many independent factors encountered by disseminating cells in different microenvironments. Future studies building on the notions and questions raised here might help build a more comprehensive picture of this process with the hope of translating this knowledge to clinical applications. The majority of clinically used tests depend heavily on cancer cell–intrinsic factors in defining prognosis. Deciphering microenvironment-based mechanisms that regulate DTC fate would complement such tests and perhaps provide more accurate predictions with respect to different breast cancer subtypes. One interesting question is whether a differential ability to bypass dormancy or survive in specific secondary sites accounts for the differential relapse incidence and tropism between these subtypes. Our understanding of how the current standard-of-care therapies affect the dormant DTCs and micrometastases is still in its infancy and more research is needed to bridge these gaps. Finally, the studies discussed here and by others (219) are shedding light on how lifestyle habits and comorbidities can affect DTC fate by manipulating the microenvironment(s). These aspects will be important to consider in the quest to translate the science of dormancy to clinical practice with the goal to design more holistic and effective clinical management strategies for patients with breast cancer.

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