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Review

Clinical and molecular complexity of breast cancer metastases

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ABSTRACT

Clinical oncology is advancing toward a more personalized treatment orientation, making the need to understand the biology of metastasis increasingly acute. Dissecting the complex molecular, genetic and clinical phenotypes underlying the processes involved in the development of metastatic disease, which remains the principal cause of cancer-related deaths, could lead to the identification of more effective prognostication and targeted approaches to prevent and treat metastases. The past decade has witnessed significant progress in the field of cancer metastasis research. Clinical and technological milestones have been reached which have tremendously enriched our understanding of the complex pathways undertaken by primary tumors to progress into lethal metastases and how some of these processes might be amenable to therapy. The aim of this review article is to highlight the recent advances toward unraveling the clinical and molecular complexity of breast cancer metastases. We focus on genes mediating breast cancer metastases and organ-specific tropism, and discuss gene signatures for prediction of metastatic disease. The challenges of translating this information into clinically applicable tools for improving the prognostication of the metastatic potential of a primary breast tumor, as well as for therapeutic interventions against latent and active metastatic disease are addressed.

1. Introduction

Tumor metastasis is a major clinical challenge accounting for the vast majority of cancer related deaths. Although only 5–10% of newly diagnosed breast cancer patients present with cancer that has metastasized to distant body parts [1–3], the risk of developing metastatic disease in patients with localized primary disease following successful primary tumor resection and adjuvant therapy remains high. It is estimated that up to 30% of node-negative breast cancer patients and an even larger fraction of patients with node-positive disease will develop metastatic disease despite receiving standard treatment [1,4]. These figures and the fact that distant recurrent disease must generally be viewed as an incurable disease indicate the high clinical burden of metastatic breast cancer (MBC) and underscore the urgent demand for better strategies for clinical intervention for those more than half a million women world-wide still succumbing to this disease annually [5].

It has been recognized for some time that breast cancer dissemination is a non-random, organotropic process, originally based on Paget's theory of "seed and soil" [6]. Factors influencing the development and localization of breast cancer metastases have been identified and will be discussed in this review. Furthermore, important associations between molecular subtypes and risk as well as site/s of recurrence have emerged and will be reviewed herein. Challenges in the path to clinical translation and how recent advances in the understanding of the complexity of breast cancer metastases may inform future management of early stage breast cancer patients are addressed.

2. Tumor progression

Tumor progression from an early pre-neoplastic lesion through invasive cancer to the development of clinically detectable distant metastases may be conceived as an evolutionary process, involving multiple genetic and epigenetic alterations affecting both tumor cells and the surrounding stroma, allowing seeding of metastases at distant sites. Although the path toward metastatic colonization

Abbreviations: BBB, blood–brain barrier; BRCA, breast cancer associated; ER, estrogen receptor; CTC, circulating tumor cell; ctDNA, circulating tumor DNA; DRFI, distant recurrence-free interval; DTC, disseminated tumor cell; EMT, epithelial-to-mesenchymal transition; HER2, human epidermal growth factor receptor 2; MBC, metastatic breast cancer; PR, progesterone receptor; TNBC, triple-negative breast cancer.

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is a complex and multi-faceted process, it is also thought to be highly inefficient. The likelihood of a circulating tumor cell forming a metastatic colony in a distant organ is in fact extremely low. Most cells that leave a tumor die, often due to inability to infiltrate distant organs [7,8]. Data from preclinical animal studies have shown that less than 0.02% of circulating tumor cells can survive and have the capability to seed metastases [9]. To develop metastases, primary tumor cells must invade and escape from the complex physical barriers (extracellular matrix, basement membrane and vasculature) at the primary site, intravasate into the lymphatic or vascular system, exit it to infiltrate distant organs and continue to proliferate in this foreign milieu [10]. In this context, there exists considerable heterogeneity in the metastatic potential of individual cells within the bulk of a primary tumor [11–15].

The metastatic propensity of a tumor cell is thought to be influenced by both the cell of origin and the oncogenic alterations present in the tumor. For example, the same oncogenic mutations occurring in cells at different stages of differentiation or lineages (*e.g.* stem cells) may hence lead to distinct metastatic propensities [10,16,17]. In addition, the type of oncogenic driver mutation may also influence the ability of a tumor to metastasize [17].

2.1. Linear progression model

The question of when and how metastases spread is complex and has multiple answers. Tumor cells can adopt different evolutionary paths to seed metastases and these paths may vary within and between different tumors. Two classical models of tumor metastasis are widely acknowledged. Traditionally, it has been considered that metastatic dissemination is a "late" event, occurring when the primary tumor is large [9]. In this linear progression model, heterogeneous clones in the primary tumor undergo a sequential clonal selection process, during which subclones with metastatic propensity are selected for and undergo further mutational changes endowing them with survival advantages and the capacity to grow as overt metastases in different organs [14,16,18]. Indeed, primary tumor size is a risk factor for metastatic progression, providing indirect support for this model [19]. Moreover, early studies reporting similar gene expression signatures between metastases and their corresponding primary tumors can be interpreted as further support [20]. This concept constitutes the theoretical basis for early detection, *e.g.* mammography screening, as a tool to reduce metastatic disease. In contrast, as reported in other studies [21,22], primary tumors may already contain a gene expression profile that is strongly predictive of metastasis and poor survival, thus challenging the notion that metastatic ability is acquired late during tumor progression. Given the wide degree of intra-tumor heterogeneity, analyzing a single small biopsy from a tumor may underestimate the complexity of the molecular landscape. This factor is a limitation of most genetic studies performed so far and presents a major challenge to the interpretation of these correlations as well as to the successful development of precision medicine [23].

2.2. Parallel progression model

The parallel progression model postulates that the metastatic potential is acquired very early in disease progression, when the primary lesion is small or even undetectable. It is based on the notion that disseminated cells evolve independently of the primary tumor and that different tumor clones can be seeded in parallel to distant sites [24,25]. This model implies that cancer is a systemic disease, requiring systemic (adjuvant) treatment at an early stage for efficient eradication [25,26]. In support of this model are observations demonstrating significant genetic differences between paired primary breast cancers and lymph node metastases [27–29], as well as discordances between primary tumors and distant relapses when conventional prognostic markers (ER, PR or HER2) are assessed [30,31]. In the study by Falck et al. [30], no significant discordance in single biomarkers was observed between primary tumors and synchronous lymph node metastases. However, by combining individual biomarkers to classify tumors into molecular subtypes according to the St Gallen guidelines [32], significant discordances in molecular subtypes were revealed between the primary tumors and lymph node metastases, and the prognosis was strongly correlated with the subtype of the metastatic lymph node. Moreover, an inferior outcome has also been reported when the phenotype differed between primary and metastatic disease [33–37], suggesting that fundamental alterations in the course of dissemination occur, thereby affecting outcome.

The detection and prognostic relevance of circulating tumor cells (CTCs) in patients with metastatic breast cancer as well as in patients with early-stage disease [38,39] lends additional evidence that parallel progression may occur. Nevertheless, most metastases are generally detected years, or even decades following diagnosis and treatment of the primary tumor. From this perspective, CTCs, disseminated tumor cells (DTCs) in the bone marrow or even circulating cell free tumor DNA (ctDNA) may be more relevant for the purposes of predicting disease progression and monitoring response to treatment [40]. As such, several clinical studies have been initiated to develop and validate their potential to serve as powerful tools for non-invasive detection of early/late metastatic disease and biomarkers for response to therapy.

Irrespective of the route of progression favored by a specific tumor, it is still unclear if each metastasis originates from a single progenitor cell (monoclonal seeding) [14,18], or if polyclonal seeding, where some metastases may originate from multiple events involving a heterogeneous mix of distinct sub-clones from the primary tumor as well as clones from other metastases [41–43] is an alternate path. Gundem et al. recently performed whole genome sequencing of serial primary tumors and metastases from patients with metastatic prostate cancer and confirmed that metastases from different organ sites in the same patient had sub-clonal alterations originating from multiple distinct clones, some of which were also found in the primary tumor, suggesting that this polyclonal seeding must have arisen both from the primary tumor and from other metastases [41–43]. Regardless of the mode of progression or the origin of metastatic cancer cells, considerable advancements in the knowledge of the molecular events underlying the development of metastatic disease are required before successful treatment and prevention become a reality.

3. Genes mediating breast cancer metastasis

While many of the transforming genetic and epigenetic changes necessary for oncogenesis are also necessary for metastatic progression, the principal steps of the metastatic cascade are accomplished by four main categories of genes (reviewed in detail elsewhere [16,17,44]). Briefly, the first group, *metastasis initiation genes*, allow aggressive cells to invade the surrounding tissue, attract a supportive stroma, facilitate the dispersion of cancer cells and may also play a role in infiltrating distant metastatic niches. Several genes involved in epithelial-to-mesenchymal transition (EMT; *e.g. TWIST1*, *SNAI1*, *SNAI2*) [10,16,17,44,45], extracellular matrix degradation (matrix metalloproteinases, MMPs), hypoxia (*e.g. HIF1A*), and angiogenesis (*VEGF*) have been associated with this step. The expression of these metastasis initiation genes and their target genes in primary tumors is prognostic of poor outcome [16].

Metastasis progression genes comprise the second category and co-operate to provide tumor cells with specialized

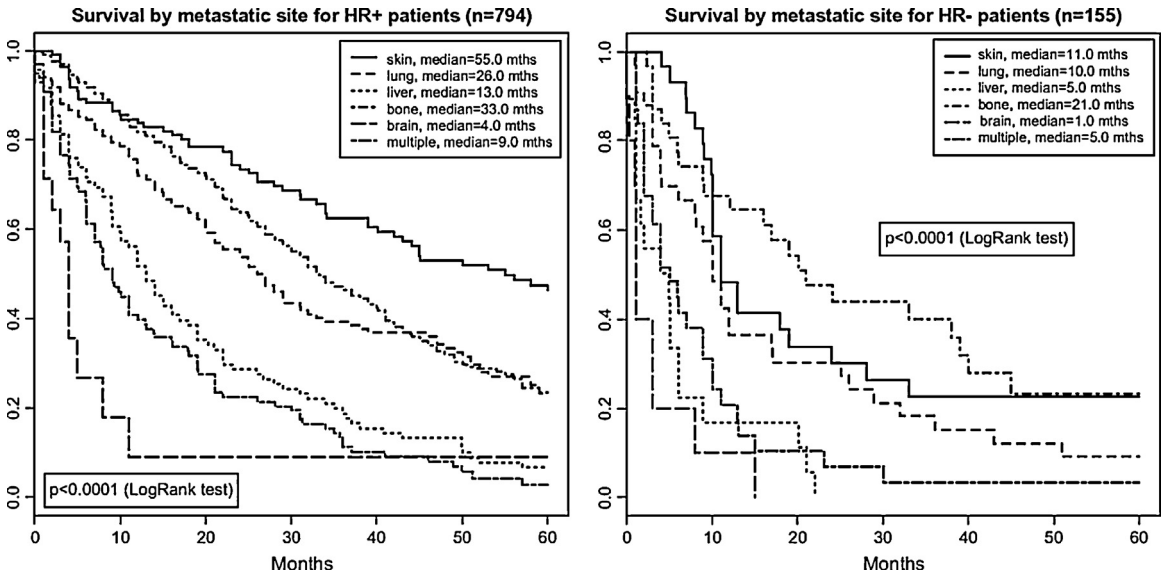

Fig. 1. Disease-specific survival from the time of diagnosis of metastatic disease for patients with hormone receptor positive (HR+) and negative (HR−) disease, respectively, according to the localization of the first metastasis recurrence.
Figure from [60] reprinted with permission from Oxford University Press.

functions required for extravasation, survival and reinitiating of tumor growth in the invaded parenchyma in a tissue-specific manner [16]. These genes may already be prominently expressed in a primary tumor, priming tumor cells for the colonization of specific distant target organs. Their expression may hence serve as site-specific prognostic markers. Evidence that metastasis progression genes are already expressed in primary tumors is provided by the repeated identification of gene expression signatures in primary tumors able to predict relapse potential [10,45–47]. The invasive front of the primary tumor is a milieu rich in tumor-associated macrophages, myeloid progenitor cells, newly generated blood vessels and cancer-associated fibroblast [48]. This stroma-rich environment is also a source of developmental and self-renewal signals including the NOTCH, and WNT signaling pathways and cytokines such as TGFβ and TNFα which support the fitness and survival of cancer stem cells. In addition, these factors may select for metastatic traits that are necessary for both local and distant metastatic niches [49]. Examples of metastasis progression genes include *PTGS2*, *EREG*, *LOX*, *ANGPLTL4* and *CLDN2* [46,47,50,51].

The third group, *metastasis virulence genes*, endows disseminated cells with the competence to overtly colonize distant sites. Frequently, there is a latency period (metastatic dormancy period) between dissemination and colonization, during which tumor cells must attain the necessary alterations to proliferate and survive in the foreign tissue [16]. This requires the activation of metastasis virulence genes, implying that their expression is only detectable in cells within the metastasis, does not provide any advantage to cancer cells at the primary tumor site, and are generally not present within the gene expression signatures predictive of the metastatic potential of primary tumors. For example, in order to establish osteolytic metastases, interleukin 11 (IL-11), vascular cell adhesion molecule 1 (VCAM-1), and parathyroid hormone-related protein (PTHrP) are essential osteoclast mobilizing factors [50,52].

Finally, there exists a group of genes called *metastasis suppressor genes*, whose functions contribute to prolonging metastatic latency and preventing metastatic cells from reinitiating growth upon infiltration of distant organs. Examples of genes in this category include cystatin E/M (*CST6*), which has been shown to suppress breast cancer bone metastases [53], retinoic acid receptor responder (tazarotene induced) 3 (*RARRES3*), which was recently identified as a potential breast cancer lung metastasis suppressor gene [54], and KiSS-1 metastasis-suppressor (*KISS1*), which has metastatic suppressor functions in breast cancer and other malignancies [55].

4. Organ-specific tropism of breast cancer metastases

Paget's "seed and soil" hypothesis for non-random metastatic spread has since its conception received support from numerous experimental and clinical studies [12,56,57]. Although the initial steps of the metastatic cascade may significantly overlap, as mentioned previously, colonization of different organs likely requires distinct traits. The circulation patterns, extravasation barriers, and potential to survive in foreign tissue constitute three key obstacles in the path of primary tumor cell colonization of distant organs. Furthermore, other capabilities are required to overcome dormancy and reinitiate growth to develop into macrometastases [10]. The unique barriers at various organ sites may affect the duration of the latency period. Estrogen receptor (ER) positive (luminal-like) tumors display protracted metastasis latency periods and frequently colonize the bone, whereas ER negative tumors display a shorter course to metastasis development and frequently metastasize to visceral organs [58–61]. This suggests that cells from ER negative primary tumors may acquire the critical metastatic traits earlier during disease progression, excluding the need for extensive adaptation after dissemination to distant sites [10].

An important factor for determining prognosis following breast cancer recurrence is tumor burden, which considers the number of metastatic lesions and the specific anatomical location of the metastases. Patients presenting with solitary (oligo) lesions survive longer than patients with multiple lesions [60]. The distant organs to which breast cancers metastasize also has clinical implications, and the site/s involved are known to affect both post-recurrence survival and overall survival [60,62–64] (Fig. 1). Overall, the most frequent sites of secondary relapse of breast cancer include the bone, lungs, liver and brain [61,65], even though a tendency toward widespread metastases to other sites has been demonstrated [57]. The preferred site of metastatic relapse has also been shown to differ between the intrinsic subtypes of breast cancer [58,59,61] (Fig. 2). The skeleton is the most common site of distant metastases, representing the first site of relapse for approximately 50%

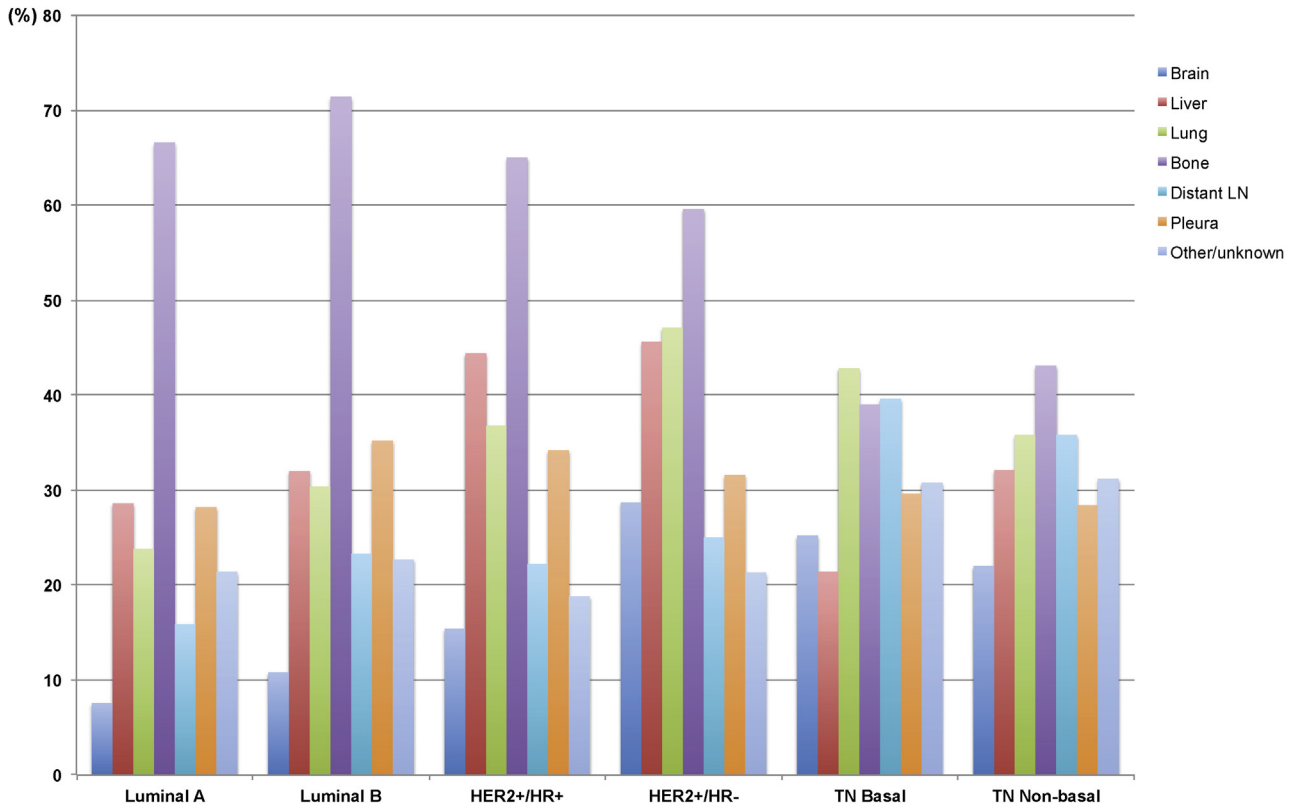

Fig. 2. Frequency (%) of site-specific metastases among 1357 patients with metastatic breast cancer based on molecular subtype. Bone metastases were most prominent in the luminal A, luminal B and luminal/HER2 subgroups. Lung metastases were less frequent in the luminal A group compared to all other groups, while high rates of brain metastases were observed in the HER2-enriched and triple negative (TN) subgroups. Liver metastases were more frequently seen in the HER2-enriched subgroups. Engagement of distant lymph nodes (LN) was observed more often in the TN subgroups.

of breast cancer patients. It is more frequent among patients with ER positive, luminal-type primary breast cancers, whereas patients with HER2 positive breast cancers frequently present with metastases in the brain, liver and lung [66]. On the other hand, lung metastases are commonly diagnosed in patients with ER negative disease, but other visceral sites, including the brain, are also common among patients with triple-negative (TNBC) or basal-like breast cancer (Fig. 2) [58,59,61]. Hence, the molecular subtype of the primary tumor may to some extent serve as a biomarker for prediction of future metastatic sites and may potentially be useful to direct disease surveillance after adjuvant treatment, although disease progression to multiple organs is often observed, with significant negative impact on patient survival. An improved understanding of the mechanisms underlying organ-specific tropism is therefore required for the development of more effective tools for prognostication, potentially targeted prevention and treatment of metastases. Examples of organ-specific breast cancer metastasis progression and virulence genes are discussed below and summarized in Table 1.

4.1. Bone metastases

Given that bone is the most common distant metastatic site for breast cancer and the third most prevalent site of cancer metastases in general, often associated with severe pain and other co-morbidities [67], the molecular determinants of bone metastases have been extensively studied and have also been recently reviewed [68]. Breast cancer cells preferentially cause osteolytic lesions in the bone. TGF-β induces osteoclasts to secrete parathyroid hormone-related protein (PTHrP), tumor necrosis factor α (TNFα), and cytokines (including interleukins 1, 6, 8 and 11) which prompt osteoblasts to release RANKL (the ligand for the receptor activator of nuclear factor-κB [RANK]), culminating in the stimulation of osteoclast differentiation [50,68–70]. Osteoclasts in turn demineralize bone, thereby releasing growth factors such as bone morphogenic proteins, IGF1, and TGFβ from the bone matrix that support cancer cell proliferation. Other proteins important for mediating the specialized functions necessary for cancer cells to home to and colonize the bone include cytokines (CXCR4, CXCL12 and TGFβ), VCAM-1, NF-κB, JAGGED1, SRC, SPP1 (also known as osteopontin, OPN), MMP1 [50,71–75], integrin (avb3) [76–78], cadherins (*e.g.* cadherin 11) [79], and adrenomedullin [80]. Recent studies implicate the enzyme lysyl oxidase (LOX), secreted by breast cancer cells under hypoxia, in inducing bone lesions specifically in patients with ER negative breast cancer [81] and the disruption of heterotypic adherens junctions (hAJs) involving cancer-derived E-cadherin and osteogenic N-cadherin to abolish bone metastatic niche-conferred advantages [82]. Furthermore, the expression of miRNAs miR-141, miR-219 [83] and MiR-34a [84] were also reported to inhibit bone metastases in a xenograft mouse model. miR-16 and miR-378 expression were also correlated with bone metastasis burden and may serve as potential therapeutic targets and clinical biomarkers of bone metastases [83].

4.2. Lung metastases

The development of lung metastases has been linked with several genes encoding cytokines or secreted products that support transendothelial migration from circulation into the lung parenchyma [85–88]. The extracellular matrix components

Table 1
Examples of organ-specific breast cancer metastasis progression and virulence genes.

Organ site	Gene	GO annotation/function	Ref.
Brain			
	ST6GALNAC5	Protein glycosylation	[46]
	COX2 (PTGS2)	Prostaglandin biosynthesis	[46,96]
	HBEGF	Cell motility	[46]
	SERPINI (neuroserpin)	Serine proteinase inhibitor	[97]
	L1CAM	Cell adhesion	[97]
	PLG (plasminogen)	Proteolysis	[97]
	Cathepsin S	Proteolysis	[94]
Liver			
	CLDN2	Tight junction	[51,99,100]
	CCL9	Chemokine	[98]
	CX3CL1	Chemokine	[98]
Lung			
	COX2 (PTGS2)	Prostaglandin biosynthesis	[47,86]
	SPARC	Cell adhesion	[47,86]
	POSTN	Extracellular matrix protein in stem cell niches	[88]
	VCAM1	Cell adhesion	[47,85]
	MMP1	Extracellular matrix remodeling	[47,86]
	MMP2	Extracellular matrix remodeling	[47,86]
	EREG	HER/ErbB family ligand	[47,86]
	ANGPTL4	Vascular regulator induced by TGFβ	[87]
	MTDH	Transcription coactivator activity	[86]
	TNC	Extracellular matrix protein in stem cell niches	[47,88]
	ID1	Inhibitor of DNA binding and transcriptional activation	[85]
	RARRES3	Protein binding/phospholipase A2 activity	[54]
	TGFB1 (TGFβ)	Cytokine	[87]
Bone			
	SDF1 (CXCL12)	Chemokine	[71,72]
	LOX	Extracellular matrix crosslinking enzyme	[81]
	IL11	Cytokine	[50]
	CTGF	Connective tissue growth factor production	[50]
	MMP1	Extracellular matrix remodeling	[73]
	ADAMTS1	Disintegrin and metalloproteinase	[73]
	JAG1 (JAGGED1)	Notch receptor ligand	[74]
	SRC	Tyrosine protein kinase activity	[71,72]
	NF-κB	Transcriptional activation of immune genes	[75]
	CXCR4	Chemokine receptor for SDF1	[69,71,72]
	SPP1 (OPN)	Integrin-mediated signal transduction	[50]
	PTHLH (PTHrP)	Osteoblast development	[70]
	VCAM1	Cell adhesion	[78]
	TGFB1 (TGFβ)	Cytokine	[70]

tenascin C (*TNC*) and periostin (*POSTN*) have been shown to play important roles in the lung metastatic niche of experimental breast cancer models [88]. TNC is found in stem cell niches and is necessary for stem cell functions. Through its action on musashi and other factors, it enhances NOTCH and WNT signaling in cancer cells. Its expression in primary breast tumors was associated with increased risk of lung relapse [47,88]. *POSTN* was also shown to be important for initiating lung metastatic growth in mouse models by mechanisms augmenting NOTCH and WNT signaling [89]. Furthermore, decreased *RARRES3* expression was recently associated with lung metastatic potential in primary breast cancer [54]. *RARRES3* downregulation facilitated the adhesion of the tumor cells to the lung parenchyma. Thus, *TNC* overexpression or *RARRES3* downregulation may serve as potential biomarkers to identify patients at high risk of lung relapse. Other noteworthy factors, which function to mediate lung metastasis, include TGFβ, epiregulin, cyclooxygenase-2 (COX-2, gene name *PTGS2*), matrix metalloproteinases (MMP1 and MMP2), angiopoietin-like 4 (*ANGPTL4*), ID1, MTDH and VCAM1 [85–88,90,91].

4.3. Brain metastases

HER2 positive breast cancer is associated with a markedly increased risk of brain metastases compared with other subtypes, and current effective therapies controlling extra-cranial metastatic HER2 positive breast cancer has made this even more evident [92,93]. The severe attrition of metastatic cells in the brain and the relatively late diagnosis of brain metastases in the clinical disease course argue that CTCs face major hurdles in colonizing this organ. The blood–brain barrier (BBB) is composed of endothelial cells, astrocytes and pericytes which present a daunting structure for tumor cells to penetrate in order to seed metastases in the brain. Cathepsin S (*CTSS*) expression in primary breast tumors was found to be inversely correlated with brain metastasis-free survival and its expression in experimental xenografts was shown to facilitate transmigration of the BBB through cleavage of tight junction proteins. Inhibition of CTSS prevented the formation of brain metastases in this model [94]. Kodack et al. recently reviewed the preclinical and clinical advances in the understanding of the development breast cancer brain metastases and possible preventive and treatment strategies [95]. Evidently, cancer cells utilize specialized mechanisms to traverse the BBB, and other key molecular mediators of this process have been identified [46,96,97]. Brain metastatic breast cancer cells express high levels of anti-plasminogen activator serpins, including neuroserpin (*SERPINI1*) and serpin B2, to prevent the generation of plasmin, which suppresses metastasis formation [97]. COX-2, the epidermal growth factor receptor (*EGFR*) ligand HBEGF, the α2,6-sialyltransferase *ST6GALNAC5*, L1 cell adhesion molecule (*L1CAM*), neuroserpin and plasminogen (*PLG*) in cooperation with astrocytes, pericytes and other cell types in the brain microenvironment act as mediators of cancer cell passage through the BBB, collectively contributing to vascular co-option and survival of breast cancer cells in the brain [46,96,97].

4.4. Liver metastases

Despite being a very common site of breast cancer recurrence, characterization of the molecular determinants of liver colonization is lagging behind. Similar to the bone, brain and lungs, chemokines secreted by breast cancer cells and their cognate receptors have been associated with breast cancer liver recurrence in a number of studies. CCL9 and CX3CL1 were strongly and uniformly elevated in liver-metastatic breast cancer cells in mice [98]. CCL9 secreted by cancer cells is important for recruiting immature myeloid cells which produce MMP2 and MMP9, and CX3CL1 is required in tumor-associated macrophages to support angiogenesis which is required for successful colonization of the liver [98]. The expression of the tight junction protein claudin 2 (CLDN2) was found to be significantly elevated in breast cancer liver metastases, as well as in primary tumors with an increased predilection to metastasize to the liver [51,62,99]. Functional evidence characterizing CLDN2 as a breast cancer liver metastasis progression gene that endows circulating breast cancer cells with enhanced capability to adhere, survive, and proliferate in the hepatic microenvironment has been demonstrated [51,99,100]. CXCR4 and its ligand (CXCL12, also known as SDF1), cadherins,

integrins, and other claudins have also been implicated in the development of breast cancer liver metastases [51,62,98–101].

Taken together, the common denominator of all these studies is that overt colonization of specific organs critically depends on very intricate interactions between disseminated cells and specific stromal components in metastatic niches. Tumor cell derived proteases and their regulators principally undergo stage-specific changes in expression during metastatic seeding and outgrowth in different organs, whereas stromal-derived genes are predominantly regulated in a tissue specific manner.

5. Prognostic factors and predictive gene signatures

Although a complete understanding of the etiology of MBC is still actively being researched, several factors have been linked with an elevated risk of developing MBC. Conventional factors associated with an increased risk of breast cancer recurrence and which are widely included in prognostic and treatment design decisions in the adjuvant setting include age at the time of primary tumor diagnosis [102,103], TNM staging (tumor size, nodal status, *de novo* distant metastatic disease [104–107]), tumor histological grade (Nottingham histological grading system [108]), ER and progesterone receptor (PR) status [109–113] and amplification of the human epidermal growth factor receptor 2 (HER2) gene [113–116]. Uncontrolled proliferation is one of the hallmarks of cancer [117,118] and significantly influences the efficacy of cancer chemotherapeutics and radiotherapy. The expression of the nuclear protein Ki67 has been shown to correlate with the proliferative rate of tumor cells and this biomarker has shown independent prognostic utility in primary breast cancer, especially among patients with ER positive tumors [115,119,120].

The risk of breast cancer recurrence and mortality may also vary over time. While patients with ER negative breast cancer usually develop metastases early (within 5 years), approximately 50% of recurrences in patients with ER positive disease will occur following a relatively protracted time period (often beyond 5 years [109]). Also, while the risk of distant recurrence peaks in the first 2–3 years after primary tumor diagnosis and thereafter declines among patients with TNBC [121], recurrence risk remains relatively constant over time among patients with ER positive breast cancer [122]. Recent advances in genetic profiling of tumors have extended our understanding of the biology of breast cancer and more importantly has led to the development of several prognostic gene signatures (reviewed in [123]) which in combination with conventional prognostic factors are routinely used in the clinic for the selection of patients with ER positive breast cancer who are at the highest risk of early disease recurrence (within 5 years), and who may benefit from extended endocrine treatment.

Several phase III trials have been also been conducted to prospectively evaluate the effect of extended endocrine treatment with tamoxifen (ATLAS [124]) or aromatase inhibitors (MA.17 [125], NSABP-B33 [126] and ABCSG [127]) beyond 5 years for ER positive breast cancer, and it is becoming more apparent that extended endocrine therapy can reduce the risk of late recurrences in the decade after completing 10 years of endocrine treatment, *i.e.* in the second decade after initial breast cancer diagnosis [124,126,127]. Unfortunately, identifying patients who are at increased risk of developing a late recurrence remains a challenge. Likewise, sparing patients at low risk from unnecessary treatments, that may also be associated with serious adverse effects, remains an unmet clinical need. A few retrospective studies have validated the applicability of multi-gene signatures for predicting late recurrence risk in ER positive breast cancer. The PAM50 risk-of-recurrence (ROR) score (a Nanostring® assay, which based on PAM50 intrinsic subtypes, tumor size and proliferation, and incorporating the number of positive lymph nodes, categorizes patients into a low, intermediate or high risk group) was shown to differentiate patients with respect to risk for late recurrence beyond conventional prognostic factors [128,129]. EndoPredict, a qRT-PCR-based score combining the expression levels of eight proliferative and ER signaling genes, stratified patients at risk after 5 years of endocrine treatment into a low risk group with an absolute risk of 1.8% of developing late distant metastases at 10 years of follow-up. Inclusion of the clinical risk factors tumor size and nodal status improved the prediction of late recurrences further [130]. In addition, the Breast Cancer Index (BCI, a qRT-PCR assay based on the five-gene molecular grade index (MGI) and the *HOXB13*/*IL17B* ratio) [131] and the 70-gene microarray prognosis signature MammaPrint® [132] also provided additional prognostic information for the identification of late distant recurrences in selected subgroups. These predictors may be helpful in identifying patients for extended therapy after five years of initial endocrine treatment. However, larger prospective randomized trials investigating the extent to which these signatures predict the benefit of extended endocrine treatment need to be conducted.

To address the impact of receiving adjuvant chemotherapy for breast cancer on survival following distant recurrence, a large meta-analysis was recently performed by Tevaarwerk and colleagues [133]. This meta-analysis of post distant disease recurrence survival included 13,785 breast cancer patients who had received adjuvant chemotherapy within 11 trials coordinated by the Eastern Cooperative Oncology Group (ECOG) over a period of 30 years. A marginal improvement in survival over the past 30 years was noted for the entire population, but this effect was not maintained when the analysis was adjusted for distant recurrence-free interval (DRFI) and hormone receptor status. In fact, survival improved over time only in those patients with hormone receptor negative disease with a DRFI ≤ 3 years, which may be largely attributed to the introduction of targeted therapy for patients with HER2 positive tumors. In general, the availability of new therapeutic agents has not broadly translated into improved survival for many women who develop distant recurrences after adjuvant chemotherapy. There hence remains a critical need for the development of more effective therapies for patients with MBC.

6. Predicting site/s of metastasis

Predicting the future metastatic site/s of a primary breast cancer is multifaceted and challenging. As previously pointed out, molecular subtypes of breast cancer may provide marginal metastasis site-specific prognostic information, but more accurate site-specific biomarkers are needed. Multigene signatures that may be useful in predicting breast cancer relapse to the bone, lung and brain in clinical tumor cohorts have been published [46,47,50,71,87,134], but further studies to validate the site-specific predictive potential of these signatures are required. As mentioned previously, several metastasis progression genes have also shown potential as independent site-specific prognostic biomarkers. A recent study by Wolf and colleagues aiming to find gene expression modules active in breast cancer subpopulations identified three independent gene modules enriched for extracellular matrix (*i.e.* stroma) genes which showed significant associations with the site of recurrence [135]. Furthermore, we have recently shown that claudin-2 expression in primary tumors is predictive of liver-specific recurrence [62]. Prospective studies are warranted to validate the analytical validity and clinical utility of these biomarkers.

7. Longitudinal heterogeneity of conventional prognostic and treatment predictive markers during disease progression

Following the plethora of changes tumor cells must undergo to seed clinically detectable metastases, the level of intra- and inter-tumoral heterogeneity between primary tumors and metastases observed at the molecular level is not surprising. Remarkable heterogeneity in the mutational spectrum, copy number alterations, transcriptomes and epigenomes between primary tumors, CTCs, DTCs and metastases has been revealed through high-throughput molecular profiling studies. More recently, studies exploring this inter-tumor heterogeneity proposed a novel classification of breast cancer that integrates genomic and transcriptomic information to classify primary tumors into 10 subtypes with distinct clinical outcomes [136,137]. Furthermore, the heterogeneity within individual subtypes, *e.g.* the TNBC subtype, can also be deconstructed to identify stable sub-groups with distinct molecular, pathological and clinical features [138].

There is a growing recognition that intra-tumor heterogeneity within the same patient is clinically relevant and the status of treatment predictive biomarkers may also evolve during tumor progression. Discordant expression of ER, PR and HER2 between primary breast tumors and their matched metastatic lesions has been extensively reported (recently reviewed in [31]). Furthermore, a meta-analysis showed that the rates of discordance for ER, PR and HER2 status were 20%, 33% and 8%, respectively [139] with the conversion to negative receptor status at recurrence, on average, higher than the positive conversion (24% *vs.* 14% for ER, 46% *vs.*15% for PR, and 13% *vs.* 5% for HER2) [139]. In a recently conducted prospective analysis, conversion rates determined at local laboratories were found to be higher than those determined centrally (21% *vs.* 13% for ER, 35% *vs.* 28% for PR and 16% *vs.* 3% for HER2, respectively) [140]. Fewer studies have however reported on the effects of biomarker discordance on decisions regarding treatment choice and overall survival. In a pooled analysis of the prospective BRITS and DESTINY studies [33], biopsy results altered management in 14.2% of cases, with rates ranging between 17-31% within prospective clinical trials [140–143]. Of note, modification of therapy was more common when there was gain of receptor expression, while other retrospective studies have associated loss of ER expression with an inferior post-recurrence survival [34–37] but this has not been prospectively investigated.

Tumor heterogeneity of breast cancer therefore represents a severe impediment to the successful clinical management of breast cancer. The significant molecular/genetic differences within individual cancers, between primary cancers and their paired metastases and potentially between metastases within the same patient, have serious implications for treating metastatic disease. Therapeutic management of MBC still largely depends on historical data for treatment predictive biomarkers assessed in the primary tumor. This paradigm is now gradually changing and biopsies of metastases are routinely collected whenever possible for reassessment of biomarkers, in compliance with clinical guidelines [1,144]. The decision regarding when and how to re-assess biomarkers and which metastasis to sample is however not an easy one, since many patients are often diagnosed with multiple metastases. Obtaining a metastasis biopsy is an invasive and costly measure and is somewhat restricted in that it may only provide a snapshot of the tumor molecular profile at a given time or organ site, but has become clinical routine in patients with a clinical or radiological suspicion of metastatic disease. There is clear evidence supporting the prognostic value of analyzing biomarkers in a metastatic biopsy, but to our knowledge any formal proof of an ameliorated outcome if treatment is directed based on information from the metastasis is not yet available. This does of course not exclude the possibility that such benefit may in fact exist. Intuitively, it is illogical to treat a manifest ER-negative breast cancer lesion from a surgically removed ER-positive primary tumor with an endocrine agent. Furthermore, biopsies of suspected breast cancer metastases regularly identify an unrelated malignant or even benign disease [34], with obvious fundamental implications for the treatment of the individual patient. Taken together, obtaining a biopsy at time of first metastasis diagnosis is highly recommended for diagnosis verification, to guide treatment and for research purposes. Re-assessment of resistant clones may be considered thereafter [31]. A possible strategy to reduce the risks associated with repeated biopsies may be to use non-invasive liquid biopsies instead. Analysis of CTCs can be used to predict/monitor treatment response for personalizing treatments [38,39] and emerging data indicate that genomic alterations in solid cancers can be reliably characterized through massively parallel sequencing of circulating ctDNA released from cancer cells into plasma [40,145,146]. These methods are however also technically challenging and expensive and do not fully capture the molecular heterogeneity of metastases. Further translational research is clearly warranted in this important field.

8. Treatment in early and metastatic breast cancer

The main goal in the treatment of breast cancer is to prolong survival and to maintain or improve quality of life for the patient. To achieve this, a large palette of anti-neoplastic medical treatments is at hand for use in the adjuvant – *i.e.* treatment of presumed micro-metastatic disease – and metastatic setting – *i.e.* treatment of macro-metastatic disease [1,144,147,148]. Conventional chemotherapy aims to eradicate proliferating cells, whereas targeted treatments including endocrine manipulations and HER2-directed treatments are mainstay in ER positive [149,150] and HER2 positive disease respectively [151], affecting central signaling mechanisms in tumors expressing these specific targets. An increasing number of other pathway directed drugs including mTOR inhibitors [152,153], PI3 kinase inhibitors [154,155] and cyclin dependent kinase inhibitors [156] have been or are currently being developed, often in combination with endocrine treatments or chemotherapy. Anti-angiogenic drugs [157,158] and drugs interacting with bone metabolism are being used, as well as PARP inhibitors that seem to have a specific role in the treatment of *BRCA* deficient tumors with decreased DNA repair capabilities [159–161]. With the exception of the bone directed treatments with bisphosphonates in postmenopausal women with early breast cancer [162,163] and the RANK ligand antibody denosumab [164], none of these medical treatments specifically aim at the treatment of a specific metastatic site. Instead, they typically exert their actions more generally toward features distinguishing tumor cells from normal cells, in most cases proliferation. In some instances, companion diagnostics indicating potential responsiveness to the drug at hand are available; in most cases however, such biomarkers are unfortunately lacking.

9. Clinical translation

The development of successful strategies to treat and possibly prevent metastases will depend on even deeper understanding of the complexity of the multi-step processes of metastasis development, including the interplay between cancer cells and the local/distant microenvironment. Given the evidence supporting the parallel progression model, in which dissemination occurs in the early stages of disease progression, developing targets for the initial steps of metastasis is challenging. Also, while mutational and transcriptional landscapes of primary tumors and metastases may be

fairly similar overall, anti-metastatic treatment may need to target fundamentally different mechanisms than standard chemotherapeutics, which are generally anti-proliferative and therefore most efficiently eliminate rapidly growing cells. The extensive latency period often observed in *e.g.* ER positive breast cancer patients implies that the DTCs present during this time are dormant and not primed to establish secondary tumors in the absence of certain molecular or environmental cues. Dormant cells also generally have low metabolic and proliferative activity, rendering them insensitive to conventional anti-cancer drugs. Understanding the signals that affect DTC turnover, and the properties required for these cells to maintain a viable state despite latency, should provide valuable clues for therapeutic intervention against minimal residual disease, and in addition, metastasis progression and virulence genes may constitute the most viable targets for efficient anti-metastasis treatment. In addition, as metastatic relapse is often associated with resistance to therapy, especially in the case of targeted therapies, this will require extensive molecular and functional understanding of resistance mechanisms that likely are unique to each drug/target. Ironically, targeted adjuvant therapy may, while controlling systemic disease, favor metastatic colonization of specific organs; *e.g.* the increased incidence of brain metastases in patients with HER2 positive breast cancers treated with the monoclonal antibody trastuzumab [92,93].

Our knowledge of mediators of organ tropism in breast cancer metastasis has increased tremendously in the past decades, and the identification of tissue specific prognostic signatures might provide more tailored treatment options. Nevertheless, while these studies provide mechanistic insight into organ specific metastasis, the vast majority are based on genetically or molecularly engineered animal models. Improved models, reflecting the natural (spontaneous) course of metastasis development as well as the selective pressure caused by (adjuvant) treatment will be required for the realization of viable anti-metastatic treatment modalities. Therapies that target the mechanisms that render dormant micrometastases active will also be required. Efficacious cancer treatment will need to combine multiple anti-metastatic drugs with cytotoxic chemotherapy in the adjuvant setting. If micrometastases, whether dormant or active, are on the move between dormant niches and sites of metastasis, combination treatments will be required to prevent further reseeding and growth at metastatic sites. Interaction with host factors may also play a role. Examples include associations with inherited factors leading to tumors of a specific subtype (*e.g.* lobular breast cancer in carriers of inherited mutations in the gene encoding E-cadherin (*CDH1*)), or general DNA repair deficiency (*e.g.* the *BRCA* genes), subtypes with specific metastatic propensities. More recently, is has been shown in a recent meta-analysis that adjuvant treatment with bisphosphonates seems to preferentially benefit postmenopausal women with breast cancer rather than premenopausal [163], highlighting the effect of host mechanisms.

Given the molecular and clinical complexity of breast cancer metastases outlined herein, it is clear that the design of effective drugs, therapeutic approaches, and clinical trials involving anti-metastatic agents face a number of challenges. So, what are the conceptual shifts that will be needed for this development? Which changes can we envision? Liquid (in early and advanced stage disease) or metastatic (in advanced stage disease) biopsies will likely become mandatory in clinical work-up. The profile of a patient's primary/metastatic tumor or liquid biopsy could include not only histopathological or genetic determinants of prognosis or treatment prediction, but also a molecular snapshot indicating metastatic propensity, a measure of how adept the cells are with respect to metastatic functions, adding crucial information to a prognostic framework. If preventative measures are to succeed in the clinic, methods to identify predisposed patients are necessary, and this will entail identification of the expression of biomarkers in primary tumors and/or systemic metastases. Further, cancer therapy might be dictated by the metastatic site/s, and not only by the specific tissue (and subtype) of origin. A current example of a site specific metastasis targeting treatment is the use of bisphosphonates or denosumab (an anti-RANK antibody), or both, to treat bone metastases originating from *e.g.* breast cancer. Drug regimens for patients with cancer might include multiple drugs targeting different metastatic sites and seeding among sites. Taken together, the emerging ability to identify tissue tropic biomarkers and the maturing of the field of predictors of prognosis hold promise to eventually allow oncologists to direct treatment plans to those patients and tissues most at risk. There is now hope for achieving the ultimate goal in cancer treatment - curing metastatic disease.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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