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Metastatic Organotropism of Breast Malignancy: Molecular Mechanisms and Heterogeneity of Bcscs

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ABSTRACT
Breast cancer is one of the most-common female malignancies with a high risk of relapse and distant metastasis. Metastasis accounts for 90% of breast cancer mortality. TheBreast cancer cells preferentially metastasize to specific organs, known as "organotropic metastasis", which is regulated by subtypes of breast cancer, host organ microenvironment,cancer cells-organ interactions and interaction with immune cells. Breast cancer stemcells (BCSCs) are a small population of breast cancer cells with tumor-initiating ability, which participate in regulatingdistant metastasis in breast cancer. We investigated the heterogeneity of BCSCs according to biomarker status, epithelialor mesenchymal status and other factors. Based on the classical "seed and soil" theory, we explored the effectof BCSCs on the metastatic organotropism in breast cancer at both "seed" and "soil" levels, with BCSCs as the "seed"and BCSCs-related microenvironment as the "soil". Understanding the role of BCSCs heterogeneity and molecular mechanisms for regulating metastatic organotropism in breast cancer would provide a new insight for the diagnosis and treatment of advanced metastatic breast cancer.

I. INTRODUCTION

Breast cancer (BC) is one of the most-common malignant tumors in females worldwide and functions as the leading cause of cancer-related death [1]. In spite of the rapid development of medical technologies, many BC patients still bear the burden of a poor prognosis due to the occurrence of relapse and metastasis. It was reported that 20 - 30 % of BC patients suffered from metastasis after early diagnosis and basic anti-tumor therapies [2]. Moreover, BC patients with metastasis had a remarkably decreased 5-year survival rate of approximately 26 % [3].However, distant metastasis of BC was recognized to display organotropism, including brain, lung, liver and bone,which represented different symptoms, prognosis and treatments [4]. Bone metastasis was the most frequent BC metastatic event while BC patients with bone metastasis endured bone damages and severe pains, exhibiting the 5-year overall survival (OS) rate of 22.8 %. Lung metastasis with chest tightness or dyspnea displayed the 5-year OS rate of 16.8 %, liver metastasis with emaciation or fatigue showed the 5-year OS rate of 12.5 % while brain metastasis had a worse 5-year OS rate of 12 % with the symptoms of decreased vision, aphasia or balance disorder [5]. Therefore, it is worthy to understand the potential mechanism of the metastatic organotropism in BC, which deserves further investigation. Breast cancer stem cells (BCSCs) are a small population of breast cancer cells with typical biological features, including self-renewal, multipotent differentiation and tumor-initiating, which play an important role in mediating tumor relapse, metastasis and resistance to chemotherapy or radiotherapy [6]. It was demonstrated that BCSCs exhibited apparent heterogeneity and plasticity, which were of great importance and became a research hotspot in recent years. With regard to heterogeneity, BCSCs can be further classified into different subtypesaccording to various biological factors, for example biomarkers, epithelial/mesenchymal status and so on.Besides, the plasticity of BCSCs allowed for the reversible transition between different BCSCs subtypes, such as the transition between epithelial and mesenchymal status,which was observed in the process of BC distant metastasis[7]. Most recently, an increasing number of researches have indicated the potential relationship between BCSCs and distant metastasis of BC. BCSCs were found to mediate the process of BC distant metastasis through different biological steps, consisting of stemness maintenance in primary tumor, invading and surviving in blood circulation and colonization in distant organs [8]. However, whether BCSCs also take part in the regulation of metastaticorganotropism in BC is still unclear and deservesfurther investigation. The classical metastatic "seed and soil" theory was proposed in

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1889 and clarified the association between tumor cells and host organs [9]. Based on the "seed and soil" theory, we tried to investigate the effect of BCSCs on the metastatic organotropism of BC at both "seed" and "soil" levels, with BCSCs as the "seed" and BCSCs-related microenvironment as the "soil", which would provide a novel insight for the diagnosis and therapies of advanced metastatic BC patients.

Heterogeneity of BCSCs

It was reported that BCSCs displayed high heterogeneityamong BC patients, which played a significant rolein BC recurrence and metastasis, consisting of locationin tumor, biological characteristics, tumor-initiatingcapacity, genetic differences and so on. Based on recentresearches, we classified BCSCs into different types,mainly according to their biomarker status, epithelialor mesenchymal status and other biological factors(Table 1).

Table 1 Heterogeneity of BCSCs

Heterogeneity		Biological characteristics	Genetic characteristics
Biomarker status	CD24- CD44+ BCSCs	Tumor invasive edge; Highly invasive; [10] Great tumor-initiating capacity:100 cells [11]	Over-expressed genes:IGFBP1, ST8SIA2, PLD5, SCG5, MYOT; KEGG enrichment:focal adhesion, PI3K-AKT signaling [12]
	ALDH+ BCSCs	Center of tumor; Highly proliferative; [10] Great tumor-initiating capacity:500 cells [13]	Over-expressed genes:WNT2,IGF1,DLL1; KEGG enrichment:ribosome, oxidative phosphorylation, proteasome; [12] Mutation:BRCA1 mutation [14]
	CD24- CD44+ & ALDH+ BCSCs	The greatest tumor-initiating capacity:20 cells [13]	-
Epithelial/ mesenchymal status	Epithelial-like BCSCs	Resemble luminal stem cells of normal mammary gland; Identified by ALDH+; Highly proliferative; Mediate colonization of metastatic foci [10]	Up-regulated MET-related genes:CDH1, OCLN, CLDN [15]
	Mesenchymal-like BCSCs	Resemble basal stem cells of normal mammary gland; Identified by CD24- CD44+; Highly invasive; Mediate tumor invasion into blood circulation [10]	Up-regulated EMT-related genes:VIM, ZEB1, ZEB2 [15]

BCSCs breast cancer stem cells

Biomarker status

Classical biomarkers of BCSCs included CD24, CD44and ALDH1. CD24 is a glycosylated protein connectedto the cell membrane, which is responsible for regulatingcellular adhesion and metastasis [16]. CD44 is a transmembraneglycoprotein located on cell surface, whichcan bind various components in extracellular matrix, takingpart in cell adhesion, interaction and migration [17].ALDH1, one member of aldehyde dedydrogenase family,has the ability to oxidize retinol to retinoic acid, participatingin regulating self-renewal and maintenance ofBCSCs [18].According to biomarker status, BCSCs can be classifiedinto three types: CD24 - CD44+ BCSCs, ALDH+BCSCs and BCSCs expressing both CD24- CD44+ andALDH+. The biological characteristics of three types ofBCSCs are various as follows. CD24- CD44+ BCSCs arelocalized at the tumor invasive edge, staying quiescentwith highly invasive characteristics while ALDH+ BCSCsare located at the center of tumor with highly proliferativecharacteristics. In addition, BCSCs expressing bothCD24- CD44+ and ALDH+ are recognized as highlypurified BCSCs, exhibiting the greatest tumor-initiatingcapacity [10]. With regard to the tumor-initiating abilityin immune deficient mice, the number of CD24- CD44+BCSCs was 100 cells, compared with 500 cells in ALDH+BCSCs, while BCSCs expressing both CD24- CD44+ andALDH+ phenotypes only needed 20 cells to generatetumors, indicating its most remarkable stemness features[11, 13].Moreover, it was reported that gene expression signaturesvaried a lot between CD24- CD44+ and ALDH+BCSCs groups. The most over-expressed genes containedIGFBP1, ST8SIA2, PLD5, SCG5 and MYOT in CD24-CD44+ BCSCs group, compared with WNT2, IGF1and DLL1 in ALDH+ BCSCs group. Besides, as demonstratedin KEGG

pathways, differentially expressed genes(DEGs) were enriched in focal adhesion and phosphatidylinositol3-kinase-AKT signaling in CD24- CD44+BCSCs group while DEGs of ALDH+ BCSCs group wereinvolved in ribosome, oxidative phosphorylation and proteasome[12]. Meanwhile, Heerma van Voss found thatBRCA1 mutation could lead to a differentiation block ofBCSCs and BRCA1 related BC patients were more likelyto have ALDH+ BCSCs [14].Furthermore, many researches reported that clinicopathologicalfeatures and survival status showed differencesamong three type BCSCs. The larger amount ofCD24 - CD44+ BCSCs was associated with higher possibilityof lymph node metastasis while ALDH+ BCSCswere correlated with microvessel density and estrogenreceptor expression [19, 20]. Considering histologicaltypes, medullary and metaplastic breast cancersexhibited remarkably increased frequency of BCSCswith CD24- CD44+ and ALDH+ [21]. Besides, BCSCsexpressing both CD24- CD44+ and ALDH+ were
related with worse progression-free survival (PFS) andcould serve as an independent prognostic factor in somesubgroups of triple negative breast cancer [22].

Epithelial/mesenchymal status

It is recognized that the reversible transformation of epithelialcells and mesenchymal cells plays a significant rolein regulating the progression of breast cancer. The epithelial-mesenchymal transition (EMT) is defined as thetransition from epithelial cells to mesenchymal cells, withreduced cell-cell contacts, loss of polarity and cytoskeletonchanges, responsible for enhanced possibility oftumor metastasis, whereas mesenchymal – epithelialtransition (MET) exhibits reversible biological behaviors, suggesting high proliferative capacity of tumor cells forcolonization in metastatic foci [23].According to the epithelial or mesenchymal status,BCSCs can be classified into two types: epithelial-likeBCSCs and mesenchymal-like BCSCs. Mesenchymal-likeBCSCs were characterized as enrichment of EMT-relatedgenes, including VIM, ZEB1 and ZEB2 while upregulationof MET-related genes was discovered in epitheliallikeBCSCs, containing CDH1, OCLN and CLDN [15].In addition, it was demonstrated that epithelial-likeand mesenchymal-like BCSCs shared similar biologicalcharacteristics separately with luminal and basal stemcells in normal mammary glands. Based on markersCD49f and EPCAM, the heterogeneity of normal mammarygland cells was classified into four types, consistingof EPCAM+ CD49f- epithelial cells, EPCAM+CD49f+ luminal progenitor cells, EPCAM- CD49f+stem cells and EPCAM- CD49f- stromal cells. As wasreported, EPCAM+ CD49f+ luminal progenitor cellswere enriched for epithelial-like BCSCs while EPCAMCD49f+stem cells exhibited high proportion of mesenchymal-like BCSCs.Moreover, gene expression profilingindicated that epithelial-like BCSCs could be recognizedby expression of ALDH+ while mesenchymal-like BCSCscould be identified via CD24- CD44+ expression in tissue,cell lines and primary xenografts of breast cancer[10].As is known, the plasticity of BCSCs allowed thereversible transition between epithelial-like and mesenchymal-like status, suggesting the potential functionof BCSCs for regulating metastatic behaviors of breastcancer. The matrigel invasion assay indicated that mesenchymal-like BCSCs displayed more invasive propertiesthan epithelial-like BCSCs. According to the experimentresults, theories were proposed that mesenchymal-likeBCSCs mediated tumor invasion into blood circulationand could resist from anoikis apoptosis whereasepithelial-like BCSCs from niches in distant metastaticorgans exhibited high proliferative properties, promotingcolonization of metastatic foci [10]. In the meantime,many potential mechanisms were discovered to mediatethe plasticity of BCSCs. For example, the lack of miR-200c/141 cluster could promote the generation of mesenchymal-like BCSCs via increasing HIPK1 expression,thus enhancing lung metastasis of breast cancer [24].Besides, many BCSCs-related signaling pathways werealso reported to participate in mediating the process ofEMT, thus regulating the metastatic behaviors of breastcancer [25].

Other factors related with the heterogeneity of BCSCs

Apart from the mentioned factors, BCSCs can also beclassified into various types according to other importantbiological factors. Leth-Larsen R indicated that CD24-CD44+ triple-negative breast cancer cells could be furtherclassified into two types: mesenchymal/basal B andluminal/basal A types. Compared with mesenchymal/basal B type, luminal/basal A type exhibited more typicalbehaviors of BCSCs, for example mammosphere formation,chemotherapy resistance and so on [26]. In addition,due to alternative splicing, BCSCs marker CD44was divided into two splice isoforms: CD44 standardsplice isoform (CD44s) and CD44 variant splice isoform(CD44v). CD44s was positively associated with the

genesignatures of BCSCs while CD44v showed the inversetendency. Besides, the switching from CD44v to CD44sthrough splicing factor ESRP1 could promote BCSCsproperties [27]. Moreover, Mannello F. reported that amajority of BCSCs displayed marker CD49f and combinationof CD24-CD44+ and EpCAM/CD49f could beapplied as a novel marker to identify BCSCs subgroupswith high mammosphere forming capacity [28]. With regard to BCSCs marker ALDH, 9 out of 19 ALDH isoforms displayed aldehyde dehydrogenase activity with distribution differences. For example, ALDH1A1 was enriched in cytosol and nucleus, ALDH1A3 was found in cytosol while ALDH2 was located in mitochondria [29]. Meanwhile, Vaillant F found that marker CD61/beta3 integrin could recognize a potential BCSCs population with high capability for tumorigenesis in MMTVwnt-1 tumors [30]. According to Wong NK, in spite of the significance of Notch signaling to BCSCs, BCSCs could still be divided into Notch-dependent and Notchindependent groups. When blocking the Notch signaling, Notch-independent BCSCs group still possessed tumorinitiating capacity [31]. Furthermore, it was proposed by Gyan E that racial heterogeneity of BCSCs played an important role in their effects on clinical outcomes ofbreast cancer patients. Compared with BCSCs in Caucasians, CD24-CD44+ BCSCs of Asians were explored to significantly influence PFS and OS of breast cancer patients [32].

Effect of BCSCs on the metastatic organotropism in breast cancer

Despite combination of advanced therapies, many BC patients still possess a worse prognosis due to relapse and metastasis. It is well known that metastatic BC patients always exhibit the organotropism in the process of distant metastasis, including brain, lung, liver and bone. Moreover, metastatic BC patients with different distant metastatic organs always suffer from different symptoms, therapeutic schedules and survival prognosis, which highlights the importance of investigating the underlying mechanism in the organotropism of breast cancer. Most recently, many researches revealed that there was a potential association between BCSCs and the metastatic organotropism of breast cancer. According to the classical “seed and soil” theory, which was proposed in 1889 to describe the correlation between tumor cells andhost organs, we also tried to explore the effect of BCSCs on the organotropism of breast cancer at the “seed” and “soil” levels respectively, with BCSCs as the “seed” and BCSCs-related microenvironment as the “soil”. Effect of BCSCs on the metastatic organotropism as “seed”It is recognized that BC patients with different molecular subtypes always displayed apparent metastatic organotropism. At the meantime, BC molecular subtypes were reported to be associated with the heterogeneity of BCSCs. Therefore, we propose a hypothesis that the heterogeneity of BCSCs may contribute to the metastatic organotropism in breast cancer, which agrees with the“seed” model of BCSCs and deserves further investigation(Fig. 1) (Table 2).According to molecular subtypes, breast cancerpatients can be classified into four main subgroups,including luminal A, luminal B, human epidermal growthfactor receptor 2 (HER2) enriched and triple negativesubtypes. Recent researches demonstrated that molecularsubtypes of breast cancer are associated with the heterogeneityof BCSCs, consisting of proportion, molecularmarkers, epithelial or mesenchymal status and so on. Asreported by Ricardo S, luminal cell lines displayed highlevels of CD24, low levels of CD44 and low ALDH1 activitieswhile HER2-OE cell lines showed enhanced ALDH1activities and Basal/mesenchymal cell lines had low CD24expressions and high CD44 expressions [35]. Besides, Xuindicated that basal-like subtype possessed higher CD44expression with more tendency of epithelial – mesenchymaltransition, compared with luminal subtype of breast cancer [40]. Moreover, as to Kong, serum level of CD44 in triple negative subtype was remarkably higher than that in luminal subtype, which could function as an independent prognostic factor in breast cancer [38]. Based on immunohistochemistry analysis of CD24 and CD44 expression in 50 breast cancer patients, Idowu MO also suggested that CD24-CD44+ BCSCs played a significant role in triple negative subtype of breast cancer [39]. In addition, Tsukabe M found that ALDH+ BCSCs weremore likely to overlap with HER2-positive tumor cells while luminal A subtype displayed low ALDH1 activities [36]. Similar with Tsukabe M, Park SY discovered that the frequency of ALDH1-positive cells was higher in HER2+breast tumors than luminal breast tumors [37]. Apart from the association between molecular subtypes of breast cancer and the heterogeneity of BCSCs, many researches also identified the significantAt the meantime, Eroles P recognized that luminal A and B subtypes displayed the highest incidence of bone metastasis while luminal B subtype also showed a high rate of liver metastasis [34]. Moreover, compared with luminal A subtype which had the lowest metastatic risk, Kennecke H also indicated that the HER2 enriched subtype showed a higher

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metastatic rate of lung, brain and liver while the basal-like subtype had a higher metastatic rate of lung, brain and distant nodes. Furthermore, median survival time from first distant metastasis varied a lot among different molecular subtypes of breast cancer, with luminal A patients of 2.2 years, luminal B patients of 1.6 years, HER2 enriched patients of 0.7 year and basal-like patients of 0.5 year [33]. As mentioned above, different molecular subtypes of breast cancer exhibited both heterogeneity of BCSCs andmetastatic organotropism of BC and we thus suppose that the heterogeneity of BCSCs may contribute to the selectivity and targeting of distant metastatic organs in breast cancer. The role of BCSCs for mediating the metastatic organotropism of BC is still under research and urges for further investigations.

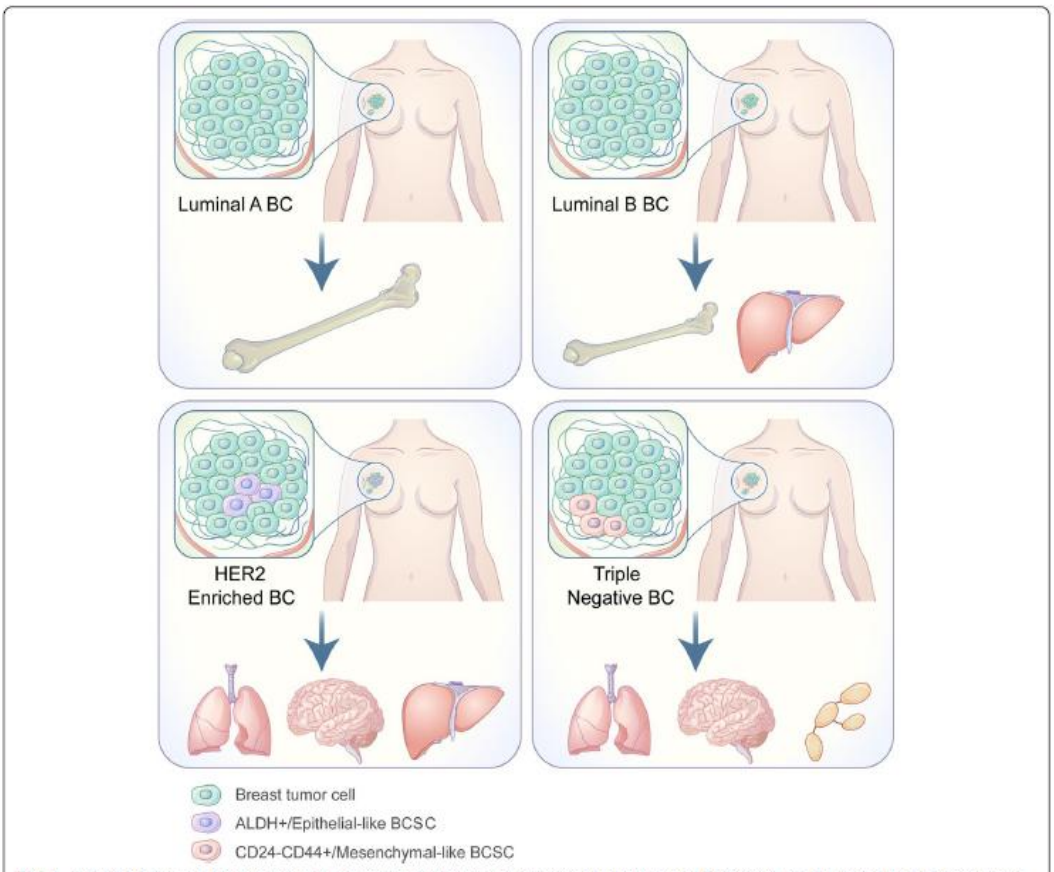

Fig. 1 Different molecular subtypes of breast cancer have different metastatic organotropism and BCSCs features. Luminal A breast cancer with a low proportion of BCSCs tends to bone metastasis. Luminal B breast cancer with a low proportion of BCSCs tends to metastasis of bone and liver. HER2 enriched breast cancer with a high proportion of ALDH+/Epithelial-like BCSCs tends to metastasis of lung, brain and liver. Triple negative breast cancer with a high proportion of CD24- CD44+/Mesenchymal-like BCSCs tends to metastasis of lung, brain and distant nodes

Table 2 Metastatic organotropism and BCSCs features in different molecular subtypes of breast cancer

Molecular subtype	Metastatic organotropism	Features of BCSCs		
		Proportion	Molecular marker	Epithelial / Mesenchymal status
Luminal A	Bone [33]	Low	-	-
Luminal B	Bone, Liver [33, 34]	Low	-	-
HER2 enriched	Lung, Brain, Liver [33]	High	ALDH+ [35–37]	Epithelial-like
Triple negative	Lung, Brain, Distant nodes [33]	High	CD24- CD44+ [35, 38, 39]	Mesenchymal-like [40]

BCSCs: breast cancer stem cells

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Effect of BCSCs- related microenvironmenton the metastatic organotropism as "soil"

Apart from the heterogeneity of BCSCs, BCSCs-related microenvironment is also identified to regulate metastatic organotropism of BC, which functions as the "soil". The BCSCs-related microenvironment is composed of cellular components and non-cellular regulatory factors. Cellular components mainly contain fibroblasts, adipocytes and immune cells while non-cellular regulatory factors consist of extracellular matrix, cytokines, physical and chemical factors. As is known, both of cellular components and non-cellular regulatory factors in BCSCs related microenvironment can influence the number or function of BCSCs by regulating signaling pathways, suggesting that the interaction between BCSCs and BCSCs related microenvironment plays an important role in BC progression, including the distant metastasis. As demonstrated by classical theories, BCSCs-related distant metastasis included three important steps: stemness maintenance in primary tumor, invading and surviving in blood circulation and colonization in distant organs. For more details, stemness maintenance in primary tumor depends on biological behaviors, like self-renewal and apoptosis, while EMT and escaping from immune response are responsible for invading and surviving in blood circulation. Hereinafter, we investigated the effect of BCSCs-related microenvironment on the metastaticorganotropism in BC (Fig. 2).

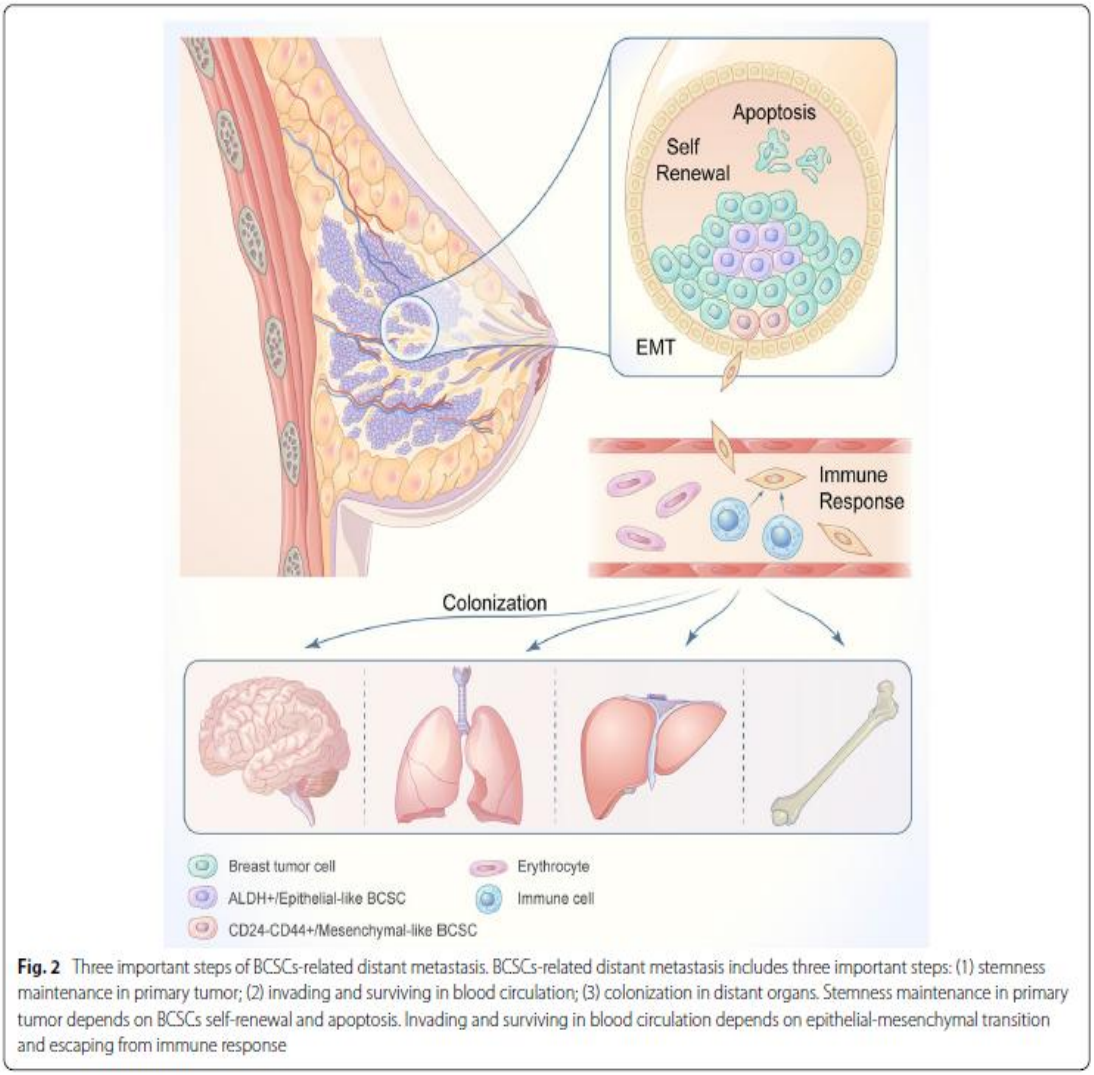

Fig. 2 Three important steps of BCSCs-related distant metastasis. BCSCs-related distant metastasis includes three important steps: (1) stemness maintenance in primary tumor; (2) invading and surviving in blood circulation; (3) colonization in distant organs. Stemness maintenance in primary tumor depends on BCSCs self-renewal and apoptosis. Invading and surviving in blood circulation depends on epithelial-mesenchymal transition and escaping from immune response

correlation between molecular subtypes and the distant metastatic sites in breast cancer, for example brain, lung, liver, bone and lymph nodes. Kennecke H demonstrated that bone served as the most common metastatic site in luminal A and B subtypes whereas the least common metastatic site in basal subtype [33]. At the meantime, Eroles P recognized that luminal A and B subtypes

displayed the highest incidence of bone metastasis while luminal B subtype also showed a high rate of liver metastasis [34]. Moreover, compared with luminal A subtype which had the lowest metastatic risk, Kennecke H also indicated that the HER2 enriched subtype showed a higher metastatic rate of lung, brain and liver while the basal-like subtype had a higher metastatic rate of lung, brain and distant nodes. Furthermore, median survival time from first distant metastasis varied a lot among different molecular subtypes of breast cancer, with luminal A patients of 2.2 years, luminal B patients of 1.6 years, HER2 enriched patients of 0.7 year and basal-like patients of 0.5 year [33]. As mentioned above, different molecular subtypes of breast cancer exhibited both heterogeneity of BCSCs and metastatic organotropism of BC and we thus suppose that the heterogeneity of BCSCs may contribute to the selectivity and targeting of distant metastatic organs in breast cancer. The role of BCSCs for mediating the metastatic organotropism of BC is still under research and urges for further investigations.

LUNG METASTASIS

Compared to other subtypes, basal-like and luminal B subtypes of breast cancer are more aggressive and show higher levels of lung specific metastasis. A new triple negative, p53 negative subtype is highly associated with lung metastasis in invasive ductal breast cancers. Compared to other metastatic lesions, lung metastatic cells have fewer roles in the lung microenvironment, but generally show aggressive growth and invasiveness. Lung metastasis molecular features Many lung metastasis signature (LMS) genes are associated with poor prognosis. From clinical data, patients with LMS expressing primary tumors are associated with primary tumor growth and high risk of metastasis and therefore exhibit worse overall survival. Genes such as epidermal growth factor receptor ligand epiregulin, COX2, MMP-1 and MMP-2 have been found to be associated with lung metastases by facilitating the angiogenesis in the tumor, releasing tumor cells into the circulation and breaching lung capillaries. Consistently, inhibition of EGFR and COX2 minimizes lung metastasis. Studies also show that protein deacetylase SIRT7 antagonizes TGFβ signalling and inhibits breast cancer lung metastasis.Lung metastasis formation also involves CSC functions, metabolic alternations and immune response. Lung metastasis can be mediated by CSCs such as CD44hi CD36+ cancer cells, which favour lipid uptake and metabolism in breast cancer and melanoma.Clinical data have shown that the presence of metastasis-initiating cells positive for CD36, a fatty acid translocase, correlates with a poor prognosis for numerous types of carcinomas. The two major biomass production (anaplerosis) pathways involved in cellular proliferation are pyruvate conversion to oxaloacetate viapyruvate (PC) and glutamine conversion to α-ketoglutarate. Cancers often show an organ-specific reliance on either pathway. Study have identified higher PC-dependent anaplerosis in breast cancer lung metastasis compared to primary breast cancers.Breast cancer cells that infiltrate the lungs can produce tenascin C (TNC), and tumor stroma can also provide a source of TNC. TNC can promote the survival and outgrowth of lung micrometastases by enhancing the expression of stem cell signaling componentsincluding musashi homolog 1, which is a positive regulator of Notch-signaling and leucine-rich repeat-containing G proteincoupled receptor 5 (LGR5), a target gene of the WNT pathway.Secretome analysis also identified cancer-specific lung metastasis secretome signatures, such as Nidogen 1 (NID1) which is associated with poor clinical outcomes. NID1 promotes lung metastasis of breast cancer by increasing cancer cell mobility and promoting adhesion of cancer cells to the endothelium, thereby disrupting its integrity, and promoting angiogenesis. RARRES3, recently characterized as a lung metastasis suppressor, regulatescancer cell adhesion and differentiation. B7x, also termed B7H4 or B7S1, is an inhibitory member of the B7 family of T cell costimulation, whose expression level is upregulated in metastatic cancers and is associated with lung metastasis of breast cancer. By using B7x knockout mice, Zhang et al. found that host B7x enables cancer cells evade local immunosuppressive responses by interacting with the innate and adaptive immune systems, including tumor associated neutrophils, machrophages and regulatory T cells.[77]

Inhibitory role of lung host tissue

Lung-derived bone morphogenetic proteins (BMPs) act as antimetastatic signals in the lung, and lung metastatic breast cancer cells need to overcome their inhibitory effect to form metastasis (Fig. 7). There are several molecules that have this ability and are considered to be metastasis promoters. A gain-of-function cDNA screen reveals that Coco reactivates dormant breast cancer cells to grow in the lung by suppressing the BMPs-mediated CSCs properties inhibition. One of the polypeptides, N-acetylgalactosaminyltransferase (GALNT), inhibits BMPs and therefore facilitates lung metastasis initiation by modulating self-renewalproperties of

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CSCs. Elevated by KRAS-PI3K-c-JUN signaling,GALNT14 also induces tumor-promoting macrophage infiltrationand exploits macrophage-derived fibroblast growth factors(FGFs). Moreover, GALNT14 serves as a prognostic marker for the pulmonary relapse in breast cancer patients.[77]

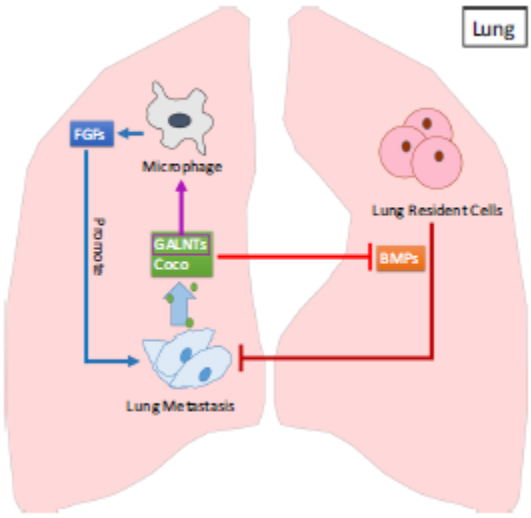

Fig 7 Lung metastatic cancer cells overcome the inhibition of lung cell-derived BMPs. BMPs secreted by lung resident cells can inhibit tumor growth by turning tumor cells into a dormancy state. Cancer cell-derived Coco and GALNTs can inhibit BMPs and reactivates dormant cancer cells to outgrowth in the lung. GALNTs support metastasis outgrowth by inducing macrophage infiltration and exploiting macrophage-derived FGFs1.[77]

BCSCs- related Lung metastasis

We investigated BCSCs-related lung metastasis according to crucial biological behaviours, which participated in BC metastasis, including self-renewal, apoptosis, EMT and immune response (Fig. 3). The BCSCs self renewal-related lung metastasis was demonstrated to be promoted by miR-31 through inhibiting the Wnt/β-catenin signaling antagonist Dkk1 [41]. Besides, benzyl butyl phthalate (BBP) activated theSPHK1/S1P/S1PR3 signaling and thereby stimulated the BCSCs self renewal-related lung metastasis [42]. Meanwhile, the BCSCs self renewal-related lung metastasis was enhanced by Id proteins via decreasing theexpression of Robo1 [43]. On the contrary, AF38469, an orally bioavailable small molecule, was discovered to weaken the BCSCs self renewal-related lung metastasis by down-regulating progranulin domain A [44]. Also, the ALDH inhibitor diethylaminobenzaldehyde (DEAB) displayed a suppressed role in BCSCs self renewal-related lung metastasis by reducing the level of HIF-2α [45]. The BCSCs apoptosis was proclaimed to be suppressed by high expression of PHGDH through up-regulating the level of NADPH, thus stimulating lung metastasis of BC[46]. Besides, the inhibition of BCSCs apoptosis caused by TUFT1 could result from the activation of Rac1/β-catenin signaling pathway, which enhanced BC lung metastasis [47]. However, miR-30 was indicated to work as a promoter in the BCSCs apoptosis via targeting bothUbc9 and ITGB3, thus preventing lung metastasis in BC [48]. In addition, a new oral compound QAP14 was disclosed to increase the expression of dopamine D1 receptor (D1DR), accordingly inducing BCSCs apoptosis and impairing BC lung metastasis[49]. The BCSCs EMT-induced lung metastasis was elucidated to be enhanced by CDK12 via activating the c-myc/β-catenin signaling pathway [50]. Moreover, theimportant role of Wnt/β-catenin signaling pathway for promoting BCSCs EMT-induced lung metastasis was proven to be supported through both BCL11A and LGR4 [51, 52]. At the meantime, AMPKα2, restrained byUBE2O, exhibited a potential role of weakening mTORC1 signaling pathway and thus accelerated the BCSCs EMTinduced lung metastasis [53]. On the contrary, the reduction of BCSCs EMT-induced lung metastasis was observed to be associated with high level of miR-1976 through targeting PIK3CG [54]. Besides, the TGF-β type I receptor kinase (ALK5) inhibitor EW-7197 served as an inhibitor in BCSCs EMT-induced lung metastasis via impairing the level of paclitaxel-induced reactive oxygen species (ROS) under the regulation of TGF-β signalling

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pathway [55]. Additionally, the role of AECHL-1,a novel triterpenoid, in repressing BCSCs EMT-inducedlung metastasis contributed to its negative regulation ofTGF-β/TNF-α [56]. With regard to immune response, it was illustrated that IL20RA could stimulate JAK1-STAT3-SOX2 signalling pathway to suppress recruitment of CD8+ T cells and natural killer cells, which inhibited immune response and thereby enhanced BCSCs-related lung metastasis in BC [57]. Nevertheless, miR-20 functioned as a suppressor in natural killer cell-associated immune response through down-regulating the level of MICA/MICB, thereby enhancing BCSCs-related lung metastasis, whichcould be restrained by all-trans retinoic acid [58]. Moreover, the natural compound emodin displayed an inhibitor in tumor associated macrophages (TAMs)-related suppressed immune response via blocking the TGF-β1 signaling pathway, which may reduce BCSCs-related lung metastasis in BC [59]. In addition, GD2-targetedchimeric antigen receptor T cells (GD2-CAR-T) was also confirmed to lead to the prevented BCSCs-related lung metastasis [60].

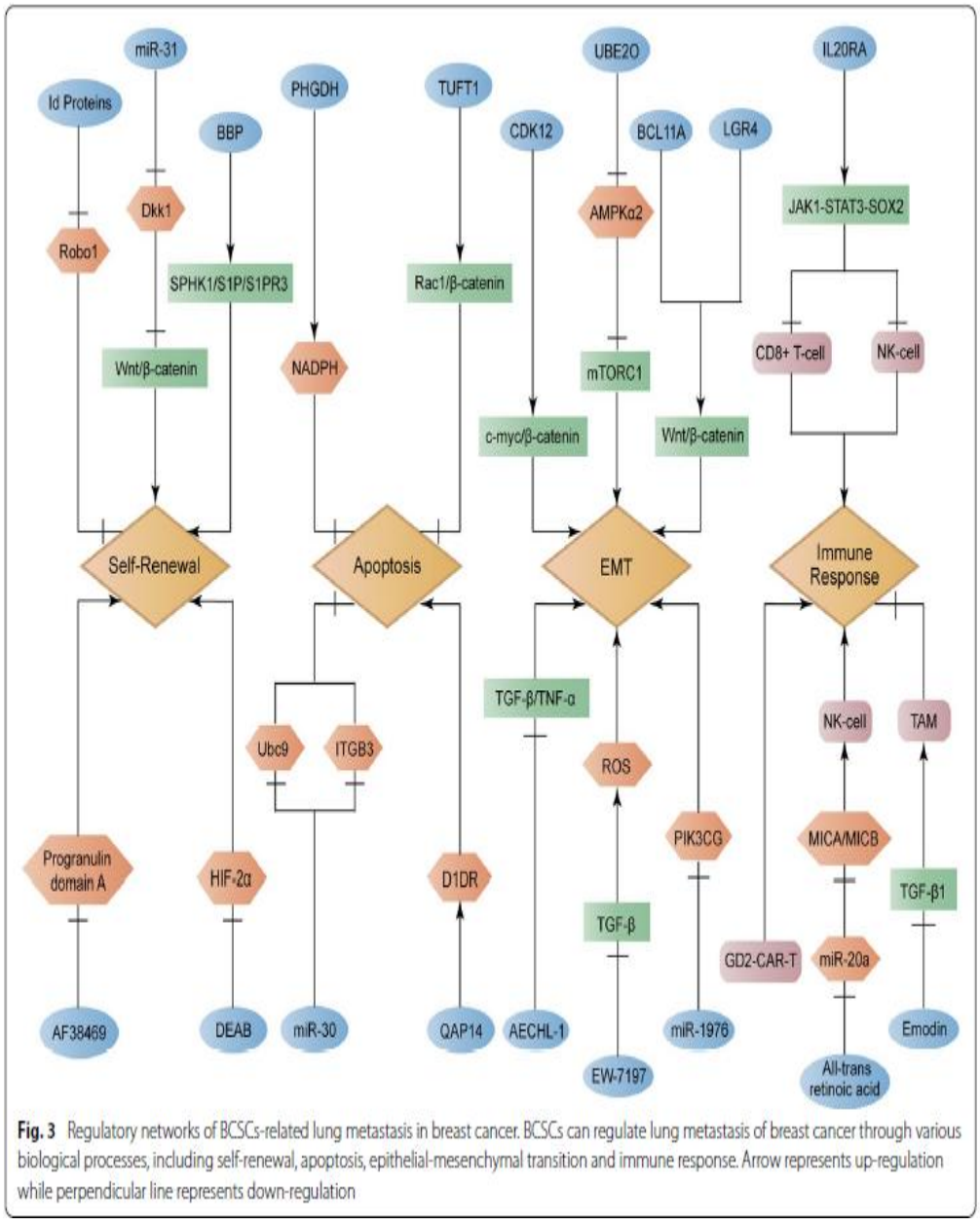

Fig. 3 Regulatory networks of BCSCs-related lung metastasis in breast cancer. BCSCs can regulate lung metastasis of breast cancer through various biological processes, including self-renewal, apoptosis, epithelial-mesenchymal transition and immune response. Arrow represents up-regulation while perpendicular line represents down-regulation

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LIVER METASTASIS

The liver is the most prevalent metastatic sites for all solid cancers and represents the second most common site for breast cancer. Liver metastases are often larger and more numerous than those of lung metastases, suggesting a metastasis-favorable microenvironment in the liver. Liver metastasis development in breast cancer patients is associated with stemness and proliferation signaling, such as beta-catenin-independent WNT signaling and Ki67, and confers a poor prognosis. Liver relapse is associated with ER expression, luminal B subtype, and is prognostic for an inferior post-relapse survival.[77]

Liver metastasis molecular features

Breast tumor cell-secreted cytokines and chemokine receptors are associated with liver metastasis. CXCR4 is the most common chemokine receptor mediating liver metastases initiation and CXCR4/CXCL12 participate in extravasation of tumor cells within the liver in a rat model. Cytokines also stimulate macrophages to produce TNFα, which up-regulates E-selectin expression, and therefore promotes cell adherence to endothelium. Moreover, dysregulation of cell adhesion molecules N-cadherin and Ecadherin contribute to breast cancer liver metastases (Fig. 8). Breast cancer cells with the high levels of N-cadherin enhance liver metastases due to N-cadherin-promoted motility and invasion.Breast cancer liver metastases maintain high levels of IL6, which decreases the metastasis-inhibitory E-cadherin levels.Integrin complexes are also involved in breast cancer liver metastasis. The α2β1 integrin complex interacts with the reticular collagen I-rich fibers in liver stroma and inhibition of α2β1 blocks the direct interactions of tumor cells with distinct matrix components and reduces liver metastasis. Claudin-2 facilitates cell/matrix interactions by increasing the cell surface expression of integrin complexes α2β1 and α5β1 in breast cancer cells.Although Claudin-2 is weakly expressed in primary breast cancer cells, it is detected in all liver metastases samples, facilitating interactions between tumor cells and primary hepatocytes.Claudin-2 expression level in liver metastasis is elevated by paninhibition of Src family kinase (c) signaling pathways. Neutralizing antibodies targeting α5β1 or α2β1 can block Claudin-2- mediated adhesion to fibronectin and type IV collagen, andreduce the ability of breast cancer cells to metastasize to the liver. Therefore, α2β1 or α5β1 complex can promote breast cancer cells metastasize to the liver in the Claudin-2 signaling pathway.The transmembrane adapter protein DNAX-activating protein of 12 kD (DAP12) can activate multiple signals for several arrays of receptors. DAP12 expression in breast cancer cells is correlated with a higher rate of bone and liver metastases as well as poor prognosis. Liver-specific homing of breast cancer cells displays unique transcriptional fingerprints, characterized by downregulation of ECM (stromal) genes.125 PPFIA1 (liprin-α1) expression can be significantly higher in the liver metastases than the primary tumors and serves as a potential poor prognostic indicator of increased metastatic relapse in ER+/N− (nodal negative) breast cancer group. β-catenin-independent WNT signaling coincides with a poor prognosis in patients with breast cancer liver metastasis.Liver-metastatic breast cancer cells exhibit a unique metabolic program compared to bone or lung metastatic cells, characterized by increased conversion of glucose-derived pyruvate into lactate and decreased mitochondrial metabolism. Pyruvate dehydrogenase kinase-1 (PDK1)-dependent metabolic reprogramming is a key regulation of metabolism and liver metastasis in breast cancer.PDK1 is specifically required for metabolic adaptation to nutrient limitation and hypoxia as a HIF1α target in liver metastatic cells. Additionally, HIF-regulated genes LOX, OPN, VEGF, and TWIST coordinate to promote breast cancer liver metastasis. LOX inhibition has no significant effects on primary tumor growth but significantly decreases lung metastases and depletes liver metastases. The quinoxaline di-N-oxide DCQ blocks breast cancer metastases by targeting the HIF1 pathway and exhibits robust antitumor activity, enhances animal survival, and reduces metastatic dissemination to the lungs and liver.[77]

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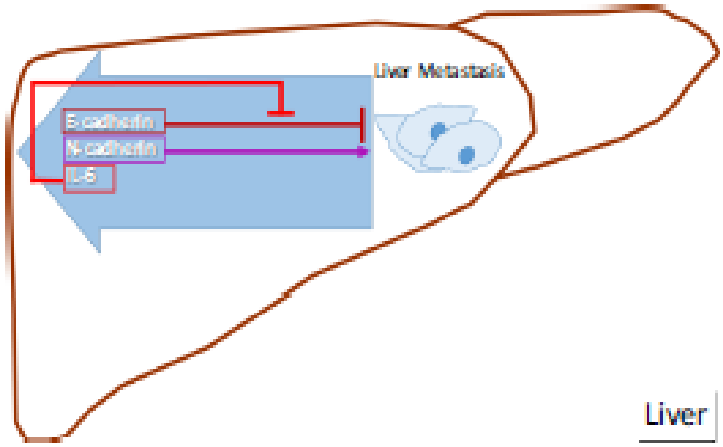

Fig 8 Dysregulation of cell adhesion molecules N-cadherin and Ecadherin in liver metastasis. N-cadherin promotes motility, invasion, and metastasis. E-cadherin suppresses liver metastasis formation, while high IL-6 levels in breast cancer liver metastases inhibit function of E-cadherin[77]

Special feature of liver metastasis based on liver biological structure

Liver is a densely vascularized organ with unique biological structure. It has fenestrated vasculatures, and the endothelium without organized sub-endothelial basement membrane. This structure allows the transportation of big molecules, and influences the interactions between cancer cells and liver microenvironment. Liver metastases can develop the suitable environment for their own growth by replacing the hepatocytes and co-opting the vasculature. However, in contrast to colorectal cancer liver metastases, which expand with concomitant hypoxiadriven angiogenesis, breast cancer liver metastases can growwithout hypoxia and angiogenesis. By using two-photon microscopy, Martin et al. examined the interaction between cancer cells and the microenvironment during early stage of breast cancer metastases and compared tumor cells in the liver and the lungs. They demonstrated that more tumor cells extravasate to the liver (56%) than the lungs (22%) 24 h after tumor cell injection. There were two subsets of lesions: a majority of lesions remained the same size, consisting of a few cells between days 5 and 12 after injection, which may not utilize the blood supply and remain dormant in the liver. Another subset formed with a patent vasculature formation that have the capacity to establish a small micrometastatic lesion in the liver microenvironment. This suggests that the same breast cancer cells can show different behavior in different host microenvironment.[77]

BCSCs- related Liver metastasis

We investigated BCSCs-related liver metastasis according to important biological behaviors in BC metastasis, including stemness maintenance and EMT (Fig. 4). It was clarified that smoothened (Smo) up-regulated the level of STAT3, accordingly promoting BCSCs maintenance related liver metastasis in BC [61]. Besides, S100A10 was discovered to participate in enhancing BCSCs maintenance-related liver metastasis [62]. In addition, combined treatment with JAK2 inhibitors (ruxolitinib and pacritinib) and SMO inhibitors (vismodegib and sonidegib) could served as a suppressor in BCSCs maintenance-related liver metastasis by simultaneously blockingJAK2-STAT3 and SMO-GLI1/tGLI1 signaling pathways[63]. Moreover, it was reported that lovastatin weakened the BCSCs EMT-induced liver metastasis through decreasing the level of cytoskeleton-associated proteins, including FLNA, TMSB10, STMN1 and so on [64]. Also, high level of PDGFRβ, which was stimulated by TWIST1, could increase the expression of FAK and Src, thus inducingBCSCs EMT-induced liver metastasis [65].

BONE METASTASIS

Bone is the most common site of metastatic breast cancer and accounts for about 70% of metastases. It is frequently associated with osteolytic type metastatic lesions due to hyperactive osteoclast-mediated bone resorption. Although all the subtypes are prone to bone metastasis, luminal subtype tumors develop bone metastasis at a much higher rate (80.5%) than basal-like (41.7%) and HER2-like tumors (55.6%).[77]

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Bone metastasis molecular features

Integrin complexes play important roles in bone metastasis of breast cancer. Study showed that integrin αvβ3 overexpression in tumor cells promotes metastasis to bone by mediating tumor cell adhesion and signal transmission for tumor progression. Fully activated integrin αvβ3 is required in the process of LPA production, which can be induced by ATX and shows growth factor-like activities. Another integrin complex α4β1 is expressed in some osteoclast progenitors, which can promote osteolytic expansion of indolent bone micrometastasis to overt metastasis by interacting with vascular cell adhesion molecule 1. Moreover,Runt-related transcription factor 2 promotes the attraction and adhesion of breast cancer cells to the bone and confers cancer cell survival and bone colonization advantages in an integrin α5- dependent manner.Cytokines, chemokines and other growth factors can also promote bone metastasis formation. Among the genes elevating osteolytic metastatic activity, the prometastatic cytokine TGFβ can stimulate the expression of osteolytic angiogenic factors interleukin-11 (IL-11) and CTGF.SMAD4 is a tumor suppressor that inhibits tumor cell proliferation, however, it is also an osteolytic metastasis promoter linking TGFβ signaling to a subsequent induction of IL-11. Both hypoxia (via HIF-1α) and TGFβ signaling activate VEGF and the CXC chemokine receptor 4 (CXCR4) to drive breast cancer bone metastases.73 Human antigenR-regulated chemokine CCL20 promotes bone metastasis in basallike TN breast cancer by elevating the secretion of matrix metalloproteinase (MMP)-2/9 and the ratio of receptor activator of nuclear factors kappa-B ligand (RANKL)/osteoprotegerin, which is critical in the "vicious cycle". Thus, CCL20 may serve as a therapeutic target in breast cancer patients with bone metastasis. Recently, a retrospective study reviewed the clinical characteristics and risk factors for bone metastases in breast cancer patients comparing to the patients without bone metastases. The results showed more axillary lymph-node metastases, high serum concentrations of cancer antigen (CA) 125, CA153, alkalinephosphatase and low level of hemoglobin are closely related to bone metastases. In order to understand the mechanisms underlying the development of distant metastases, Van de Vijver group analyzed gene expression signatures specifically associated with the development of bone metastases in breast cancer patients, and identified a 15-gene expression signature significantly correlated to the bone metastasis status of breast cancer. These 15 genes are APOPEC3B, ATL2, BBS1, C6orf61, C6orf167, MMS22L, KCNS1, MFAP3L, NIP7, NUP155, PALM2, PH-4, PGD5, SFT2D2 and STEAP3, which encode mainly membrane-bound molecules with molecular function of protein binding. The expression levels of the up-regulated genes (NAT1, BBS1 and PH-4) correlated with EMT status of the tumor.[77]

Vicious cycle: cross-talk of tumor cells and bone microenvironment

Breast cancer metastases to bone leads to bone loss by promoting bone degradation and interfering with bone remodeling.Metastatic breast cancer cells extravasate from capillaries to the bone marrow and gain the bone cell-like properties by osteomimicry that improves homing, adhesion, proliferation and survival in the bone microenvironment. More importantly, the relationship between bone resorption and tumor growth forms the "vicious cycle" (Fig. 5). Tumor-derived factors such as osteopontin (OPN), parathyroid hormone-related peptide (PTHrP), heparanase, IL-1, IL-6 and prostaglandin E2 (PGE2) enhance the osteoclasts formation and promote bone resorption. Resorbed bones release bone-derived growth factors, such as IGF1, PDGF, and TGFβ, as well as calcium that stimulates skeletal tumor proliferation. This vicious cycle accelerates bone loss and provides a fertile soil for tumor growth.Several components have been identified as master factors in this process. Tumor cells that reach in the bone microenvironment secrete PTHrP to initiate osteolysis and stimulate bone lining osteoblasts. In response, the expression of RANKL is upregulated by activated osteoblasts and binds to its receptor RANK to form RANKL-RANK signalling pathway, which is involved in activating the differentiation of preosteoclasts into activated osteoclasts, and leading to bone resorption. The activated osteoclasts subsequently degrade the bone matrix by releasing hydrogen ions to create strong acid, and also releasing proteinases such as the cathepsin-K (cat-K), MMP-9, and MMP-13.82,83 Bone degraded by osteoclasts can release TGFβ, IGF1, and other growth factors stored in the bone matrix, and these growth factors in turn stimulate tumor growth and lead to increased levels of tumor derived PTHrP.[77]

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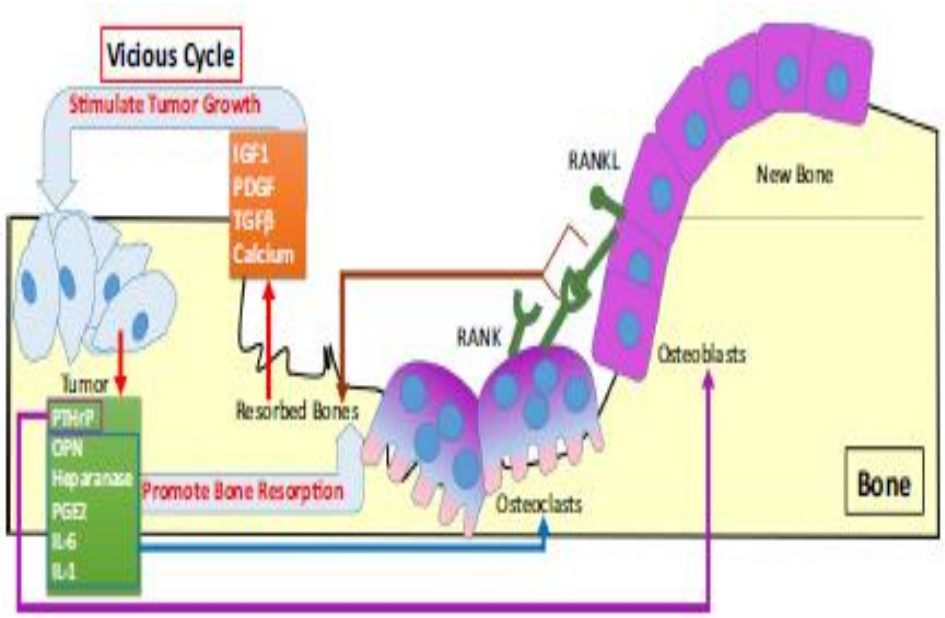

Fig-5 Vicious cycle of bone metastasis. Tumor-derived factors such as OPN, PTHrP, heparanase, IL-1, IL-6 and PGE2 enhance the osteoclasts formation and promote bone resorption.Resorbed bones release bone-derived growth factors, such as IGF1, PDGF, and TGFβ, and calcium, which in turn stimulate tumor growth.Tumor cells that reach in the bone microenvironment secrete PTHrP to initiate osteolysis andstimulate bone lining osteoblasts.In response, the expression of RANKL is upregulated by activated osteoblasts and binds to its receptor RANK to activate RANKL-RANK signaling pathway, and leading to bone resorption.[77]

BCSCs- related bone metastasis

We investigated BCSCs-related bone metastasis according to important biological behaviours in BC metastasis, including stemness maintenance, dedifferentiation and EMT (Fig. 4). It was recognized that bone-derived osteopontin (OPN) supported the BCSCs maintenance-related bone metastasis in BC via enhancing the phosphorylationof WNK-1 and PRAS40 [66]. Meanwhile, TGF-β, which was inhibited by BMP2/7 heterodimer, could strengthen the BCSCs maintenance-related bone metastasis through activating the level of Smad [67]. Additionally, high expression of CXCR4 was found to be associated with the enhanced BCSCs maintenance-related bone metastasis[68]. At the meantime, mesenchymal stem cell (MSC)- derived extracellular vesicles (EVs) were announced to strengthen the Wnt/β-catenin signaling pathway, whichpromoted the dedifferentiation of breast cancer cells into BCSCs and enhanced colonization of BC in bone marrow[69]. What's more, hypoxia-induced high expression of Jagged2 was reported to stimulate the Notch signaling pathway,thus increasing BCSCs EMT-induced bone metastasis[70]. Moreover, a natural small-molecule compound ZL170 was investigated to refrain TGF-β/BMP,which enhanced BCSCs EMT-induced bone metastasis via up-regulating Smads[71]. Furthermore, it was clarified that miR-628 could act as a suppressor in BCSCs EMT-induced bone metastasis through targeting SOS1 [72].

BRAIN/CNS METASTASIS

There are 10–30% of patients with metastatic breast cancer develop brain/ CNS metastases. Several factors associated with the increased risk of brain metastases have been identified, including young age, poorly differentiated tumors, HER2-enriched, luminal-HER2, basal-like and TN breast cancer subtypes, and four or more metastatic lymph-nodes. In most cases, brain metastasis is viewed as a late complication of disease, and happens after metastases have appeared systemically in the lung, liver, and/or bone for which few effective treatment options exist. The two main sources of brain metastases are adenocarcinomasof the lung or the breast. Brain metastases are not only associated with an extremely poor prognosis but also with neurological impairments by often affecting both cognitive andsensory functions. Brain metastasis from breast cancer show patterns of parenchymal

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brain metastasis or leptomeningeal metastasis. Parenchymal brain metastasis account for approximately 80% of all brainmetastases. Metastases to the brain parenchyma are thought to be hematogenous in origin. The co-option of the breast cancer cells with host vascular tissues is essential for tumor cells growth. For leptomeningeal metastases, breast cancer is the most common solid tumor origin. Once the tumor cells reach the leptomeninges, they may spread via the cerebrospinal fluid.[77]

Breaching of the blood–brain barrier (BBB)

To form the brain metastasis, CTCs need to breach the interface between the circulation and the brain, the BBB, and then interact with local microenvironment in order to survive and then form the metastasis colony. Breaching the BBB involves mediators of extravasation through non-fenestrated capillaries, complemented by specific enhancers of BBB crossing and brain colonization (Fig.6). BBB is composed of capillary endothelial cells backed up by basal lamina, pericytes and astrocytic end-feet. Tumor cells usually transmigrate the BBB through paracellular endothelialtight junctions. CD44, VEGF and CXCR4 can enhance this transendothelial migration process by disrupting endothelial integrity. Angiopoietin-2 (Ang-2) also increases BBB permeability by impairing ZO-1 and claudin-5 tight junctions protein structuresand can cause the subsequent colonization of TN breast cancer cells in brain. Gene expression analyses of cells with high brain metastatic activity identified COX2, EGFR ligand heparin-binding EGF-like growth factor (HBEGF), and ST6GALNAC5 as mediators of cancer cell passage through the BBB. For example, ST6GALNAC5 specifically mediates brain metastases by enhancing tumor cells adhesion to brain endothelial cells. COX2 can promote the expression of MMP1, which is the only MMP significantly correlated with brain metastasis. Furthermore, COX2 and prostaglandin activate astrocytes to release chemokine(C–C motif) ligand 7, which in turn promotes self-renewal of CSCs or tumor-initiating cells in the brain. Moreover, the BBB is responsible for the breast cancer patients with brain metastases showing fewer CTCs compared with breast cancer patients with other metastases.[77]

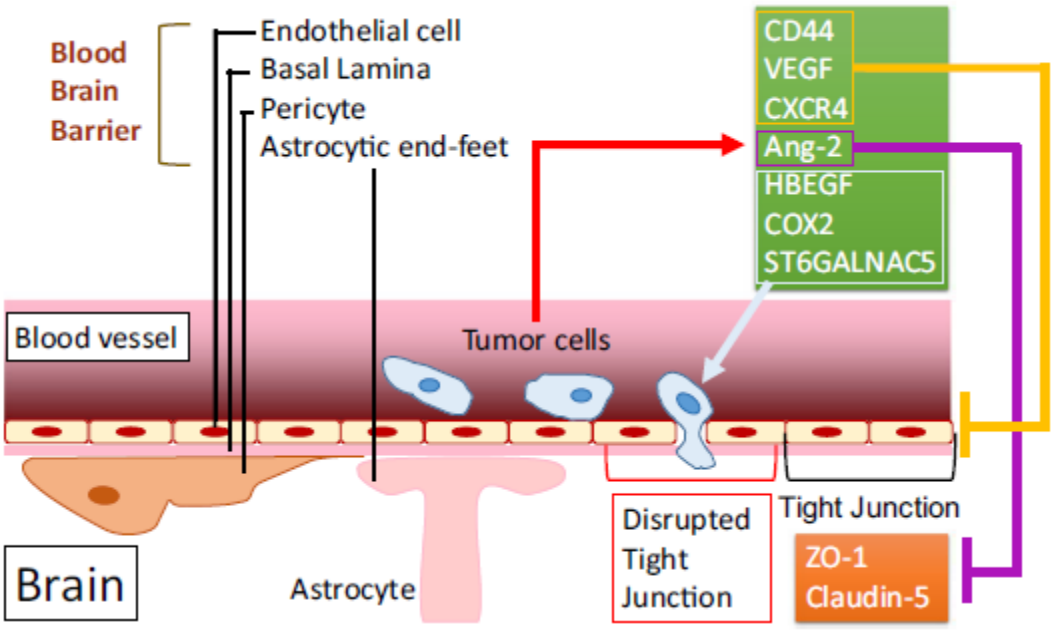

Fig 6Brain metastatic cancer cells breach the blood–brain barrier (BBB). BBB is composed of capillary endothelial cells, basal lamina, pericytes and astrocytic end-feet.CD44, VEGF and CXCR4 can enhance the transendothelial migration of tumor cells by disrupting endothelial integrity.Ang-2 increases BBB permeability by impairingZO-1 and Claudin-5 tight junction protein structures.COX2, HBEGF, and ST6GALNAC5 mediate cancer cell passage through the BBB.[77]

Brain metastasis molecular feature

We have discussed that brain metastatic cells are related with some CSC markers, such as nestin, CD133 and CD44. In comparisons of primary breast tumors with metastases, very high

frequency of hypermethylated genes are found in metastases to the bone, brain, and lung. In particular, hypermethylation of cyclin D2, retinoic acid receptor-β, and hin-1 are more frequently detected in brain metastases. In addition to HER2, HER3 overexpression is also associated with brain metastases in breast cancer patients. The primary ligand of HER3/HER2 heterodimers heregulin (HRG) is highly expressed in the human brain and is ableto induce the transendothelial migration of HER2/HER3-positive breast cancer cells across a tight barrier of brain micro vascularendothelia. Finally, MMP-9 has been identified as one of the factors partially mediating this process. Interestingly, in breast cancer cells, HRG-induced MMP-1 and MMP-9 expression is mediated through HER3-dependent pathway and cells with higher HER2 level is more aggressive than those with the lower HER2 expression. A potential signature of brain metastasis marker HER2+/EGFR+/HPSE+/Notch1+ in EpCAM-negative CTCs has been identified as high invasive and capable of generating brain and lung metastases in xenograft model.[77]

Interaction between brain metastatic cells and host cells

After tumor cells that have infiltrated into the brain in order to grow and develop into a metastatic lesion, they need to recruit blood vessels and establish the suitable metastatic microenvironment Brain-seeking metastatic cells secrete significantly more VEGFA and IL-8 than the parental cells. VEGF is a principle angiogenic factor and contributes to the outgrowth of the brain metastases.When tumor cells arrive in the brain, there is an intensive cross talk with the residential brain cells. The association between brain microvascular cells, astrocytes and neurons forms functional “neurovascular units”, and recent studies have highlighted the importance of brain endothelial cells in this modular organization. Interactions between the brain endothelium, astrocytes and neurons that may also regulate BBB functions. In breast cancer,the brain metastatic cells gain the ability to exploit the brainendogenous substrates secreted by the resident cells to facilitate the oncogenic growth. Tumor cells may show the gammaaminobutyricacid (GABA)-ergic phenotype as neuronal cells withupregulated proliferation by taking up and catabolizing GABA intosuccinate and subsequent NADH as biosynthetic source.Studies have shown that among different glial cells, astrocytesand microglia are associated with brain metastases. Localastrocytes can be activated by tumor cells and then secrete ahost of soluble proteins including IL-1, IL-3, IL-6, IFNγ, tumornecrosis factor-α (TNF-α), TGFβ, IGF1, PDF1, and other cytokines.Many of these factors, such as IL-6 and TGFβ, can function asoncogenic signals for the tumor cells. In contrast, Plasmin fromthe reactive brain stroma inhibits metastatic invasion by convertingmembrane-bound astrocytic Fas ligand into a paracrine deathsignal for cancer cells and inactivating the neuronal cell adhesionmolecule L1 cell adhesion molecule, which promotes the spreadof tumor cells and formation of large metastases. To counter the inhibitory signals, tumor cells express high levels of antiplasminogen activator serpins, including neuroserpin and serpinB2, to promote cancer cell survival and vascular co-option in brain metastasis. Co-cultured breast cancer cell lines with astrocytes exhibited astrocytes-derived factors MMP-2 and MMP-9, which induce both the migration and invasion of breast cancer cells. Microglia can also be activated by tumor cells and perform similar functions as astrocytes to promote colonization tumor cells, and this process occurs in a Wnt-dependent manner.[77]

BCSCs- related brain metastasis

We investigated BCSCs-related brain metastasis according to important biological behaviors in BC metastasis, including stemness maintenance and EMT (Fig. 4). It was confirmed that the role of miR-7 in impairing the BCSCs maintenance-related brain metastasis contributed to its negative regulation of KLF4 [73]. Besides, PCDH7 was illustrated to be refrained by the selective PLC inhibitor edelfosine and showeda potential of supporting BCSCs maintenance-related brain metastasis by stimulating the PLCβ-Ca2+/CaMKII/ S100A4 signaling pathway [74]. Moreover, combination of reparixin and paclitaxel was recognized tosuppress BCSCs maintenance-related brain metastasis via decreasing the level of CXCR1 [75]. However, the ALDH1A3 inhibitor MF-7 was elucidated to weaken BCSCs EMT-induced brain metastasis by impairing the expression of ALDH1A3 [76].

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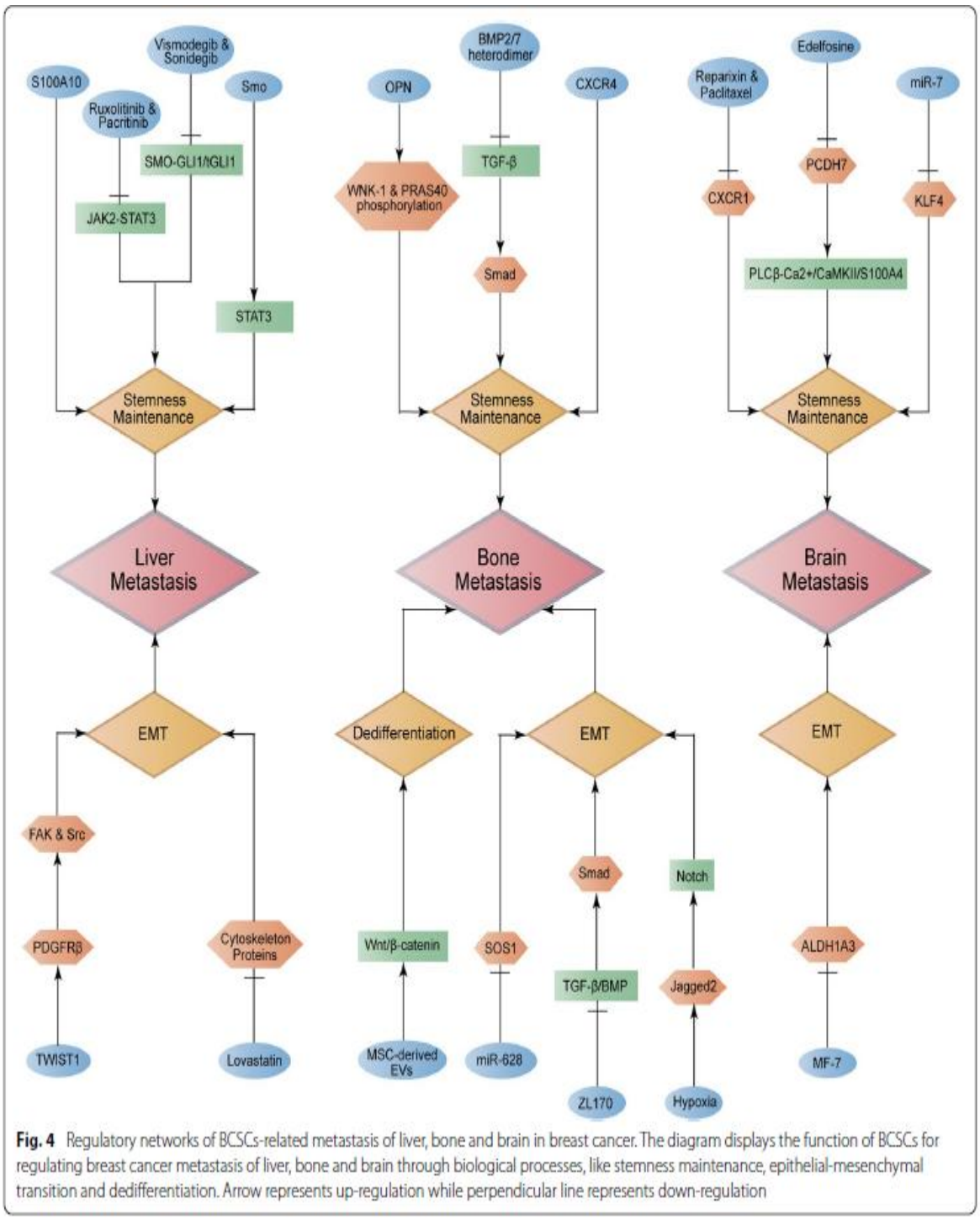

Fig. 4 Regulatory networks of BCSCs-related metastasis of liver, bone and brain in breast cancer. The diagram displays the function of BCSCs for regulating breast cancer metastasis of liver, bone and brain through biological processes, like stemness maintenance, epithelial-mesenchymal transition and dedifferentiation. Arrow represents up-regulation while perpendicular line represents down-regulation

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LYMPH-NODE METASTASIS

Lymph-node metastasis indicates a high risk of distant metastasis. Absence of lymph-node metastases correlates with low risk of distant metastasis, whereas the presence of more than four lymph-node metastases predicts very high risk of distant metastasis. It has been well known that tumor metastasis to distant sites does not occur exclusively through the axillary lymph nodes (ALD), but also through blood circulation. Therefore, the lymph-node metastatic status should be used as an indicator of the tumor cells' ability to metastasize. A correlation has been found between tumor size and the percentage of positive lymphnodemetastases. Luminal A, luminal B, luminal-HER2 and HER2-enriched subtypes of breast cancer are highly correlated with lymph-node metastases and poor outcome in the patients with ALD metastases, butnot in the patients with tumor-negative lymph-nodes. A high ratio of lymphovascular invasion and high expression of Ki67 are independently predictive of ALD metastases. Another potential biomarker, cytoplasmic chromosome segregation 1 Like is significantly associated with ALD metastases although it appears to have no regulatory effects on ALD metastases. Axillary lymph-node dissection used to be a standard surgical procedure for breast cancer since the 1800s, but has been replaced by sentinel lymph-node (SLN) biopsy, which has become the routine procedure for early breast cancer patients because of its benefits and minor side effects. When the axillary SLN has no evidence of micrometastases, the nonsentinel lymph-nodes (NSLNs) are unlikely to have metastases.Comparing to theNSLN-negative group, four kallikrein (KLK) subfamily members (KLK10, KLK11, KLK12, and KLK13) are up-regulated, while B cell antigen receptor (BCR) signaling pathway is downregulated in the NSLN-positive group. Consistently, breast cancer tissues show a higher expression of KLK10 and KLK11 than the non-carcinoma mammary glands151 and the dysregulation of KLK gene family is closely associated with endocrine-related cancer, such as prostate, breast, and ovarian cancers. Therefore, more studies are needed to confirm the role of KLK family in lymph node metastasis. It is known that the BCR signaling pathway is critical for B lymphocytes development and survival, and plays significant roles in chronic lymphocytic leukemia. However, this is the first report about the role of BCR signaling in breast cancer lymph node metastasis,
which warrants further investigation.[77]

The immune system in organotropic metastasis

The immune system contributes to each cascade of metastasis. At the primary site, it is involved with PMN formation in specific sites. For example, in a xenograft model of the human breast cancer cell line MDA-MB-231, tumor cells induce CD11b+ immune suppressor myeloid cells recruitment in the pre-metastatic lung via secretion of lysyl oxidase (LOX).49 Primary breast tumor hypoxia can also induce CD11b+/Ly6Cmed/Ly6G+ myeloid cell accumulation and reduces the NK cell cytotoxicity in the pre-metastatic lung. Moreover, recruitment of functional monocytes/macrophages by tissue factor-mediated coagulation is essential for metastatic cell survival and PMN establishment in the lungs. When tumor cells enter the circulation, immune cells also interact with tumor cells and affect the metastatic sites. Studies have suggested that neutrophils can assist metastasis of CTCs. In response to inflammatory cues, neutrophils release neutrophil extracellular traps (NETs) which can capture CTCs and support the formation ofmicrometastases. Metastatic breast cancer cells also induce neutrophils to make metastasis-promoting NETs and support lung colonization. However, tumor-entrained neutrophils inhibit metastatic tumor cell seeding to the lungs by generating H2O2, upon activation by tumor secreted CCL2 (chemokine ligand 2).The neutrophil polarization (N1 vs. N2) which is regulated by specific tumor-derived factors such as TGFβ may explain these inconsistent results, however when and where neutrophil polarization is shaped remains to be elucidated. In lung cancer studies, neutrophils promote liver metastasis via neutrophil macrophage-1 (Mac-1) mediated interaction with intercellular adhesion molecule 1 in CTCs, and interactions between adherent neutrophils and CTCs within the inflamed liver sinusoids may further increase tumor cell arrest in the liver. However, whether neutrophils play the similar roles in breast cancer metastasis warrants further investigation. T cells also participate in regulation of organotropic metastasis by expressing different proteins. A study has shown that IL-17-producing gamma delta (γδ) T cells activate the expansion and polarization of neutrophils which in turn suppress cytotoxic CD8+ T cells and promote lung metastases. T cell-expressed prolylhydroxylase proteins can create immunoregulatory environment for lung, thus facilitating tumor cell colonization and metastasis formation by limiting pulmonary type helper (Th)-1 responses, promoting Treg cell induction, and restraining CD8+ T cell effector function. Moreover, CCR4 expressing Treg cells are required

for lung metastasis by directly eliminating tumor suppressing NK cells through beta-galactoside-binding protein. During bone metastasis of breast cancer, tumor-specific RANKL expressing T cells induce pre-metastatic osteolytic bone disease and promote bone metastasis formation.Interactions between immune cells, host environment and tumor cells are essential for the organ-specific metastasis formation. For breast cancer lung metastasis, breast tumorevoked regulatory B cells promote lung metastasis by converting resting CD4+ T cells to Treg cells, which perform immune suppression role.62 Depletion of the host sphingosine-1-phosphate transporter spinster homolog 2 (Spns2) can increase the infiltration of effector T cells and NK cells into the lung, and reduce TN breast cancer cell line lung colonization and melanoma cell line lung metastasis. In addition, blocking human M2 macrophage differentiation by COX2 inhibitor reduced lung metastasis. For breast cancer bone metastasis, both clinical data and mouse model showed that silencing of IFN regulatory factor Irf7 pathways in breast cancer promotes bone metastasis through innate immune escape. Depletion of plasmacytoid dendritic cells inhibits tumor growth and prevents bone metastasis by activatingtumor-specific cytolytic CD8+ T cells.[77]

II. CONCLUSIONS

Breast cancer is one of the leading female malignant tumors with a high risk of relapse and distant metastasis. BC patients with distant metastasis always exhibit apparent organotropism, including brain, lung, liver bone and lymph node. BCSCs are a small population of breast cancer cells with tumor-initiating capacity, which participate in regulating distant metastasis of BC. However, whether BCSCs have an effect on the metastatic organotropism of BC is still unclear and deserves further investigation. In this review and summary, we investigated various mechanism of metastatic organotropism ,the heterogeneity of BCSCs according to biomarker status, epithelial or mesenchymal status and other biological factors. Then, we explored the effect of BCSCs on the BC metastatic organotropism based on the “seed and soil” theory, with BCSCs as the “seed” and BCSCs-related microenvironment as the “soil”.In this review we also investigated interaction of immune system with organotropic metastasis. Understanding These interaction with immune system can be promising for the development of targeted immunotherapy against metastatic malignancy. The understanding of these molecular mechanisms and effect of BCSCs can be future of precision medicine and targeted therapies. Thus combination of molecular mechanism, genetic study,biomarker status and immunological and biological factors with clinical relevance is critically important for further progress in this field. Integrated analysis of these will improve future precision medicine for metastatic malignancy.

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