

3. Organ-Specific Markers of Breast Cancer Metastasis to Distant Organs

To date, these features of breast cancer distant metastasis have been described in detail in the literature: clinical picture, diagnosis, biological mechanism and current approaches in the treatment of metastases to bone [4,40,41], lung [42,43], liver [44,45] and brain [46,47]. The work by Andrea R. Lim presents in detail the current relevant information on this issue [48].

However, there is scattered information on biomarkers of organ-specific metastasis in breast cancer in the literature. This section summarises the current information on biomarkers of metastasis to different target organs in breast cancer (Table 1).

Table 1. Markers of organ-specific metastasis in breast cancer.

Marker	Description	Source
BONES		
<i>P1NP, CTX, 1-CTP</i>	Patients with high serum levels of <i>P1NP</i> , <i>CTX</i> and <i>1-CTP</i> have been shown to have a high risk of metastasising to bone soon after diagnosis ($p = 0.006$, $p = 0.009$, $p = 0.008$, respectively).	[49]
<i>IL-1β</i>	In preclinical experimental mouse models, <i>IL-1β</i> inhibitors have been shown to prevent the development of bone metastases.	[50]
<i>CAPG/GIPC1</i>	The identification of <i>CAPG</i> and <i>GIPC1</i> in primary tumour samples (by IHC) was a strong prognostic indicator for the development of bone metastases of breast cancer. Cox regression analysis showed that control patients were more likely to develop first distant recurrence in bone (hazard ratio [HR] = 4.5, 95% confidence interval [CI] = 2.1 to 9.8, $p < 0.001$) and die (HR for overall survival = 1.8, 95% CI = 1.01 to 3.24, $p = 0.045$) if both proteins were highly expressed in the primary tumour.	[51]
<i>PRLR</i>	High <i>PRLR</i> expression in primary breast tumour is associated with shorter time to metastasis ($p = 0.03$).	[52]
<i>PRDX4</i>	High expression of <i>PRDX4</i> in primary breast tumour is associated with metastasis within 5 years.	[53]
<i>PAK4</i>	<i>PAK4</i> enhances the invasive potential of ER α -positive breast cancer cells in vitro and promotes metastasis in vivo. The status of the nuclear <i>PAK4</i> (<i>nPAK4</i>) scores was significantly higher in the bone metastatic breast cancer group than in the non-bone metastatic breast cancer group ($p = 2.22 \times 10^{-9}$).	[54]
<i>MAF</i>	<i>MAF</i> is a molecular target for the prevention or treatment of bone metastases because <i>MAF</i> accumulation (16q23 amplification) plays a role in bone colonisation. 16q23 gain copy number alterations (CNA) encoding the transcription factor <i>MAF</i> mediate breast cancer bone metastasis through PTHrP control. 16q23 gain (hazard ratio (HR) for bone metastasis = 14.5, 95% confidence interval (CI) = 6.4 to 32.9, $p < 0.001$) as well as <i>MAF</i> overexpression (HR for bone metastasis = 2.5, 95% CI = 1.7 to 3.8, $p < 0.001$) in primary breast tumours were specifically associated with risk of metastasis to bone but not to other organs.	[55,56]
<i>DOCK4</i>	In a triple-negative MDA-MB-231 cell line model, <i>DOCK4</i> was identified as a biomarker of bone metastasis in early stages of breast cancer. Adjusted Cox regression analyses showed that high <i>DOCK4</i> expression in the control arm was significantly prognostic for first recurrence in bone (HR 2.13, 95%CI 1.06–4.30, $p = 0.034$) (a clinical validation). High <i>DOCK4</i> expression was not associated with metastasis to non-skeletal sites when these were assessed collectively.	[57]
<i>CENPF</i>	<i>CENPF</i> promotes breast cancer metastasis to bone by activating PI3K-AKT-mTORC1 signalling and represents a novel therapeutic target for breast cancer treatment.	[58]
<i>MMP9, MMP13, TNFAIP6, CD200, DHRS3, ASS1, VIM</i>	Together, they can be considered as specific prognostic markers of metastasis to bone in primary breast cancer. The relative expression of <i>MMP9</i> , <i>MMP13</i> , <i>TNFAIP6</i> and <i>CD200</i> were significantly up-regulated ($p < 0.05$), while <i>DHRS3</i> , <i>ASS1</i> and <i>VIM</i> were significantly down-regulated in the bone metastasis compared with lung and liver metastasis ($p < 0.05$).	[59]
<i>miR-200, -128, -99a, -29b, -600, -34, -30, let-7 miRNA</i>	These miRs act as tumour suppressors and inhibit breast cancer metastasis to bone.	[60,61]
<i>miR-21</i>	Exosomal <i>miR-21</i> derived from SCP28 cells promotes osteoclastogenesis through regulation of <i>PDCD4</i> protein levels. The level of <i>miR-21</i> is significantly higher in serum exosomes of breast cancer patients with bone metastases than in other subpopulations.	[62]
<i>CXCL5/CXCR2</i>	<i>CXCL5</i> stimulates proliferation of breast cancer cells and their colonisation in bone. Inhibition of its <i>CXCR2</i> receptor with an antagonist blocks the proliferation of metastatic cells. <i>CXCL5</i> and <i>CXCR2</i> inhibitors may be effective in the treatment of tumours with metastasis to bone.	[63]

Table 1. Cont.

Marker	Description	Source
<i>RANKL/RANK</i>	<i>RANKL/RANK</i> regulates breast cancer cell migration. <i>RANKL</i> acts as a chemoattractive agent on tumour cells which overexpress one of its receptors. Blocking signalling by AMG161 (IgG1) reduces micrometastasis formation in bone marrow in vivo. Daily subcutaneous injections of 1.5 mg/kg AMG161 antibody to MDA-MB231RANK tumour-bearing animals reduced bone micrometastases and early bone marrow colonization without affecting lung micrometastasis.	[64]
<i>CXCL-12</i>	HIF signalling transduction in osteoporosis precursor cells increases blood levels of <i>CXCL-12</i> , promoting metastasis to bone.	[65]
<i>ESR1</i>	Mutations in the <i>ESR1</i> gene have been observed in bone metastases, suggesting a potential causative role. In this study, bone metastases from breast cancer (n = 231) were analysed for <i>ESR1</i> mutation. Activating <i>ESR1</i> mutations were identified in 27 patients (12%). The most frequent mutation was p.D538G (53%), no mutations were found in exon 4 (K303) or 7 (S463). Metastatic breast cancer with activating mutations of <i>ESR1</i> had a higher Ki67 labelling index than primary luminal cancers (median 30%, ranging from 5 to 60% with 85% of cases revealing $\geq 20\%$ Ki67-positive cells).	[66]
<i>ANGPTL2</i>	<i>ANGPTL2</i> increases breast cancer cell metastasis to bone by enhancing <i>CXCR4</i> signal transduction.	[67]
LUNGS		
<i>miR-106b-5p</i>	It is an independent predictor of lung metastases (based on the expression level in the primary tumour). <i>Mir-106b-5p</i> promotes lung metastasis by suppressing <i>CNN1</i> and activating the Rho/ROCK1 pathway.	[68,69]
<i>SIRT7</i>	<i>SIRT7</i> counteracts TGF β signalling and inhibits breast cancer metastases to the lung.	[70]
Tumour stem cells (TSCs) (CD44hi CD36+)	The formation of lung metastases is associated with TSC function, metabolic changes and immune response. Lung metastasis can be mediated by TSCs with CD44hi CD36+ phenotype.	[71]
<i>NID1</i>	Secretome analysis of lung metastases of breast cancer has shown that Nidogen 1 (<i>NID1</i>) is associated with poor treatment outcomes. <i>NID1</i> promotes lung metastasis of breast cancer by increasing the motility of tumour cells and promoting their adhesion to the endothelium, thereby compromising its integrity and promoting angiogenesis.	[72]
<i>EGFR</i>	EGFR inhibition successfully blocks circulating tumour cells (by immunohistochemistry) clustering and triple-negative breast cancer metastasis to the lung.	[73]
<i>VCAM-1</i>	<i>VCAM-1</i> can be considered as a potential therapeutic target in lung metastasis of breast cancer. Selective inhibition of <i>VCAM-1</i> has been successfully used to suppress the development of metastases. The experimental results showed that the SCB-loaded nanoparticles (SN) could greatly improve the oral delivery and suppress breast cancer metastasis to the lung. The cell migration and invasion abilities of metastatic 4T1 breast cancer cells were obviously inhibited by SN. Moreover, the <i>VCAM-1</i> expression on 4T1 cells was significantly reduced by SN, and the binding ratio of RAW 264.7 cells to 4T1 cells was significantly decreased from 47.4% to 3.2%. Furthermore, the oral bioavailability of SCB was greatly increased 13-fold under the effect of SN, and the biodistribution in major organs was markedly improved.	[74]
<i>DKK1</i>	In patients with breast cancer, low serological levels of <i>DKK1</i> are associated with the risk of developing lung metastases.	[75]
<i>Connexin43 (Cx43)</i>	Mice injected with Cx43-shCx43-inhibited tumour cells exhibited more lung metastases compared to parental MDA-MB-231 cells. This observation was confirmed by qPCR analysis of human 18S RNA levels in secondary metastatic sites in the lungs. Higher levels of human 18S RNA were found in the lungs of mice injected with shCx43 cells compared to the lungs of mice injected with parental MDA-MB-231 cells. This observation indicates that suppression of Cx43 increases the metastatic potential of MDA-MB-231 cells.	[76]
LIVER		
<i>Connexin43 (Cx43)</i>	Metastatic foci in the liver were almost absent in mice inoculated with parental MDA-MB-231 cells or Cx43D cells by week 9, compared to those clearly observed in mice inoculated with shCx43 cells. This result is consistent with the increased levels of human 18S RNA in the livers of mice inoculated with shCx43 cells. Inhibition of Cx43 induced metastasis of MDA-MB-231 cells to lung and liver at week 9, when the original MDA-MB-231 cells had not yet metastasised. These findings correlate with increased tumour volume and decreased survival of xenograft mice in vivo.	[76]

Table 1. Cont.

Marker	Description	Source
CXCR4/CXCL12	CXCR4 inhibition doubles the response to immune checkpoint blockers in mice with metastatic triple-negative breast cancer (TNBC). CXCL12/CXCR4-mediated desmoplasia in metastatic breast cancer promotes immunosuppression and is a potential target to overcome therapeutic resistance to immune checkpoint blockade in MBC patients.	[77]
PKD1	PKD1-dependent metabolic reprogramming is a key regulation of metabolism and metastasis to the liver in breast cancer. PKD1 is particularly required for metabolic adaptation to nutrient restriction and hypoxia as a HIF1 α target of metastatic cells in the liver.	[78]
circRNA hsa_circ_0008324 (circEZH2)	CircEZH2 enhances oncogenesis and metastasis in vitro and in vivo by activating KLF5 protein expression, which in turn activates CXCR4 transcription, leading to the initiation of the EMT programme in breast cancer.	[79]
circRNA hsa_circ_0124696 (circROBO1)	Increased expression of circROBO1 was found in liver metastases in breast cancer and correlated with poor prognosis. Knockdown of circROBO1 strongly inhibited proliferation, migration and invasion of RRM cells, whereas circROBO1 overexpression showed opposite effects. circROBO1 overexpression promoted tumour growth and metastasis to the liver in vivo.	[80]
Lyn (Src-family kinase)	The Lyn-selective kinase inhibitor, bafetinib (INNO-406), reduces claudin-2 expression and suppresses breast cancer metastasis to the liver.	[81]
PPF1A1	PPF1A1 is activated in breast cancer metastasis to the liver and is a potentially unfavourable prognostic sign of metastases development. Kaplan–Meier plotter results showed that although high PPF1A1 expression was generally associated with reduced distant metastasis-free survival in oestrogen receptor+ patients, subgroup analysis only confirmed significant association in an oestrogen receptor+/N– (node-negative) group (median survival, high PPF1A1 group vs. low PPF1A1 cohort: 191.21 vs. 236.22 months, hazard ratio: 2.23, 95% confidence interval: 1.42–3.5, $p < 0.001$), but not in an oestrogen receptor+/N+ (nodal positive) group (hazard ratio: 1.63, 95% confidence interval: 0.88–3.03, $p = 0.12$). In oestrogen receptor patients, there was no association between PPF1A1 expression and distant metastasis-free survival, regardless of Nm (mixed nodal status), N– or N+ subgroups. In bc-GenExMiner 4.0 programme using the Nottingham Prognostic Index and Adjuvant! Online-adjusted analysis validated the independent prognostic value of PPF1A1 in relation to the risk of metastasis in patients with oestrogen receptor+/N–.	[82]
ESR1, AKT1, ERBB2, FGFR4	ESR1 (20%), AKT1 (8%), ERBB2 (7%) and FGFR4 (4%) were identified as driver genes for breast cancer metastasis.	[83]
BRAIN		
PI3K	Activation of PI3K was found in a large proportion (77%) of brain metastases in patients with breast cancer, and activation of PI3K-Akt signalling in such metastases was associated with poor outcomes. Pharmacological inhibition of PI3K activity was found to attenuate the expression of PD-L1, CTLA4 and CSF1 genes, as well as the infiltration of metastatic breast cancer cells into the brains of mice.	[84,85]
CDK4 u CDK6	Abemaciclib, an inhibitor of the cyclin-dependent kinases CDK4 and CDK6, has shown potential for the treatment of brain metastases in patients with breast cancer. The combination of abemaciclib with endocrine therapy was effective in patients with HER2-negative breast cancer and brain metastases, and 38% of patients had a reduction in metastatic tumour burden.	[86]
STAT3	The STAT3 inhibitor silibinin, which penetrates the blood–brain barrier, impairs the viability of brain metastases in both mice and humans. This inhibitor is thought to block the growth of brain metastases by targeting STAT3 in tumour-associated astrocytes, thereby weakening their interaction with tumour cells and microglia.	[87]
JAK, JAK2	The JAK inhibitor ruxolitinib limits the growth of primary brain tumours and also reduces the number of tumour-associated astrocytes in mice. JAK2/STAT3 signal transduction is hyperactivated when breast cancer metastasises to the brain. Inhibition of JAK2 results in reduced brain metastasis in vivo, suggesting that JAK2 may be a promising therapeutic target.	[88,89]
COX2	COX2 can promote MMP1 expression, which is significantly correlated with brain metastasis. In addition, COX2 and prostaglandin activate astrocytes to release chemokine ligand, promoting self-renewal of tumour stem cells or tumour-initiating cells in the brain.	[90]
FABP7	FABP7 is a key regulator of metabolism in HER2+ breast cancer metastasis to the brain. FABP7 has been shown to be required for the activation of key metastatic genes and pathways, such as integrins-Src and VEGFA, as well as for the growth of HER2+ breast cancer cells in the brain microenvironment in vivo.	[91]

Table 1. Cont.

Marker	Description	Source
<i>miR-4428, miR-4480</i>	In a study of microRNAs in patients with advanced breast cancer with brain metastases, it was shown that the determination of <i>miR-4428</i> and <i>miR-4480</i> in serum may be useful as prognostic biomarkers. A total of 51 serum samples from patients with breast cancer and brain metastasis, and 28 serum samples from controls without brain metastasis were obtained. Two miRNAs, <i>miR-4428</i> and <i>miR-4480</i> could significantly distinguish patients with brain metastasis, with area under the receiver operating characteristic curve (AUC) values of 0.779 and 0.781, respectively, while a combination of <i>miR-4428</i> and progesterone receptor had an AUC value of 0.884.	[92]
<i>PLVAP</i>	<i>PLVAP</i> staining was observed not only in isolated brain microvessels but also in brain metastases in breast cancer. Immune labelling for <i>PLVAP</i> was performed in 4T1 TNBC culture, where clear expression of this protein was observed.	[93]

4. Biomarker Profile of Rare Types of Breast Cancer Metastases to Distant Organs

4.1. Gynaecological Metastases

The work by Kutasovic J.R. (2018) [94] described in detail the clinicopathological and molecular profiling of breast cancer metastasis to gynaecological organs. The study included data from 54 female patients with breast cancer diagnosed with metastasis to gynaecological tissues between 1982 and 2015. A total of 258 metastatic foci (average of five metastases per patient (range 1–11 pcs)) were reported in these 54 patients. The most frequently involved gynaecological organs were the ovaries (46/54; 85.1%), fallopian tubes (29/54; 53.7%) and uterus (20/54; 37%). The median survival of patients was only 1.95 years.

In biomarker expression analysis, *FOXA1* and *GATA3*, key regulators of transcriptional activity, were shown to be highly expressed in primary tumours. Primary tumours also demonstrated CNA with amplification of 1q, 7q, 8q, 11q, 16p and 17q and deletion of 8p, 16q, 22q and Xq (identified in more than 50% of samples). The most frequent alterations in ovarian metastases (CNA identified in more than 50% of samples) included amplifications of 1p/q, 3p, 6p, 7p/q, 8q, 12q, 15q, 17q and 19p/q and deletions on 8p, 13p/q, 16q, 22q and Xq. The most frequent amplifications were detected at loci encoding *MDM4*, *CDK6*, *FGFR1*, *MYC*, *CCND1*, *CDK4* and *MDM2*.

In analysis of targeted sequencing data from matched primary tumours and metastases, it was shown that all cases had at least one mutation in common between the primary tumour and metastases, along with unique mutations present either only in the primary tumour (e.g., *TBX3* in GM06BR) or only in the metastases (e.g., *RB1*, *TP53* in GM74LO) [94].

4.2. Metastases to the Pancreas

Genetic analyses of breast cancer metastases to the pancreas are very limited due to the rarity of metastasis to this target organ. One study is presented, which is a case report that examined biomarkers of breast cancer metastasis to the pancreas.

GATA3 expression and an *ERBB2* mutation (I767M) originating from a breast tumour were detected using immunohistochemistry and molecular diagnostics. The functional significance of this gene mutation has not been determined [95].

5. Genomic Profile of Breast Cancer Organ-Specific Metastasis

Understanding the nature of gene activity involved in metastasis has also been an important goal over the past few decades.

In addition to the development of high-throughput technologies in experimental and clinical oncology, many new prognostic gene markers (gene signatures or differentially expressed genes) that predict the risk of metastasis in patients with breast cancer have emerged [96]. In this section, current information on the study of the genomic profile (expression characteristics, active signalling pathways and CNA) of organ-specific metastasis in breast cancer is compiled.