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Metastasis: the seed and soil theory gains identity

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Abstract The metastatic spread of tumor cells to distant sites represents the major cause of cancer-related deaths. Cancer metastasis involves a series of complex interactions between tumor cells and microenvironment that influence its biological effectiveness and facilitate tumor cell arrest to distant organs. More than a century since Paget developed the theory of seed and soil, the enigma of tissue specificity observed in metastatic colonization of tumor cells begins to unfold itself. The advent of new technologies has led to the discovery of novel molecules and pathways that confer metastasis-associated properties to the cancer cells, mediating organ specificity and unique genetic signatures have been developed using microarray studies. Future clinical studies and new antimetastatic compounds aiming to improve survival of patients with metastasis will most probably be based on these signatures. This review summarizes the plethora of old and new molecules that are strongly correlated with organ-specific metastases and which provide now an identity to the theory of seed and soil.

Keywords Organ-specific metastasis · Colonization · Seed and soil · Genetic signature

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1 Introduction

Despite the significant improvement in both diagnostic and therapeutic modalities for the treatment of cancer patients, metastasis still consists the major cause of mortality being responsible for 90% of all cancer deaths [1]. Metastasis (Greek: the change in the state) is a complex phenomenon involving a series of complex biological events. Primary tumor cells continuously proliferate and their adherence to adjacent normal and abnormal cells as well as to basement membrane is decreased. Deadhesion and escape of malignant cells into blood or lymphatic vessels (intravasation) is followed by arrest through restriction in capillary beds of host tissue and exit from the circulation (extravasation). Subsequently, establishment of sufficient nutrient supply through neoangiogenesis and interaction with host stromal tissue is essential for formation of metastases. In the new environment, malignant cells will either remain in a dormant state for long time (dormant metastases) or continue to grow further [2, 3].

One particularly important issue, which has been remained unanswered for many years, is what determines in which organ tumor cells will metastasize. An extremely important observation was made in 1889 by Stephen Paget that patients with breast cancer had a predilection for metastasizing to the liver. Paget perceived this as unusual considering that other organs such as spleen could have been equally affected since they receive the same amount of blood. This enduring finding prompted Paget to develop the

theory of "seed and soil". He hypothesized that certain tumor cells (seeds) colonize selectively distant organs (soil) with a favourable environment facilitating survival of tumor cells: "When a plant goes to seed, its seeds are carried in all directions but they can only live and grow if they fall on congenial soil." Certain organs must therefore provide appropriate conditions for development of organ-specific metastases, contributing to the organ selectivity [4]. The theory had not been experimentally challenged until Sugarbakeer [5] and later Kinsey [6] retested Paget's hypothesis by ectopically transplanting various organ tissues in DBA/2 syngeneic mice and injecting them from different routes with melanoma S91 Cloudman strain [5, 6]. They reported that tumor cells metastasized specifically to lungs but not to any of the other organs transplanted. A similar observation reported by Hart and Fidler confirmed the idea that organ-selective stimuli must permit new tumor growth [7].

2 Biological identity of site-specific metastases

It became clear that metastases cannot be simply explained by anatomical or mechanical theories such as tumor cell trapping or lodgement of tumor emboli into organ vascular bed. During the past several years, biological studies have identified important molecular interactions between tumor cells (seeds) and stromal environment (soil) that dictate the tissue-specificity of metastasis formation. The discovery of numerous growth factors, chemokines, guidance molecules, signaling pathways and, more importantly, new genes provides now a new identity for organ-specific metastasis.

3 Bone metastases

Bone metastases is a major clinical problem that occurs in 70% of patients with advanced breast or prostate cancer and less frequently in other tumor types such as multiple myeloma, lung, colon, stomach, bladder, uterus, rectum, thyroid, or kidney [8]. It has been estimated that 350,000 people die with bone metastases every year in United States. Patients with bone metastasis have a low quality of life often due to severe pain, hypercalcemia, compression of spinal cord and pathological bone fractures and the prognosis of such patients still remains poor [9]. Common sites for bone metastases are the back, pelvis, upper leg, ribs, upper arm, and skull. More than 90% of all metastases are found in these locations [10].

There are mainly two forms of bone metastases, osteoblastic, found frequently in prostate cancer and osteoclastic, seen most often in breast cancer [11]. Osteoblastic metastases present a unique, consistent capacity to stimulate osteoblasts to deposit new bone together with minor bone resorption. Osteoblastic metastases are closely associated with a series of diverse biological interactions between tumor cells and bone cells where metastatic tumor cells release growth factors that in turn activate osteoblasts [12, 13]. Koeneman previously suggested that prostate cancer cells, through stimulation by osteoblast soluble factors, gain osteoblast-like properties [14].

Endothelin 1 (ET-1) has been shown to be a key factor in promoting osteoblastic metastasis [13, 15]. ET-1 is a potent vasoconstrictor peptide that has the ability to model functions of osteoblasts and stimulate expression of osteopontin and osteocalcin in osteosarcoma cells [16]. Additionally, it can induce VEGF production in osteoblasts to promote tumor angiogenesis [17]. Endothelin-induced activation of calcineurin results in translocation of NFATc1 in nucleus and inhibition of osteoblastic apoptosis [15]. Importantly, increased levels of circulating endothelin were detected in men with bone metastases from prostate cancer, indicating its secretion from prostatic cancer cells [18].

Bone morphogenetic proteins (BMP) are members of the transforming growth factor (TGF-β) superfamily. The binding of BMPs to two different types of serine-threonine kinase transmembrane receptors, type I and type II lead to the phosphorylation of SMAD1 and SMAD5 proteins [19]. Prostate cancer promotes osteoblastic activity through BMP-6 and enhances skeletal invasion [20]. BMP-7 protein, also known as osteogenic protein-1 (OP-1), modulates the osteoblastic phenotype of osteoblastic-committed cells [21] and stimulates transcriptional activation of BMP-6 but reduces BMP-2 and BMP-4 in human osteosarcoma cells [22]. Moreover, BMP-2 inhibits decorin transcription in osteoblasts increasing their differentiation.

PSA (prostate specific antigen) is a serine protease used as clinical marker for prostate cancer and can possess osteoblast-stimulating properties [23]. PSA participates in modulation of genes responsible for bone remodelling and osteoblastic differentiation favouring the development of osteoblastic metastases [24].

Wnt (wingless) pathway is also involved in osteoblastic tumor growth. Wnt proteins bind to receptors of the Frizzled and the low-density lipoprotein receptor-related proteins LRP5/6 on the cell surface. Through several cytoplasmic relay components of the Frizzled, the active signal is transduced to β-catenin, which accumulates in the nucleus and forms a complex with the T-cell factor (TCF) to activate transcription of Wnt target genes [25]. Patients with metastatic prostate cancer presented increased expression of the WNT-family ligand WNT1 in prostate cancer cells [26]. Attenuation of DKK-1, a Wnt antagonist, in PC-3 cells augmented osteoblastic activity, increasing alkaline phosphatase production and mineralization in murine bone marrow stromal cells [27].

Other important factors involved in the regulation of osteoblastic remodelling and architecture upon secretion from metastatic tumor cells include transforming growth factors beta 1 and 2 (TGF-beta 1 and 2), insulin-like growth factor 1 and 2 (IGF1/2); fibroblast growth factor (FGF); platelet-derived growth factor (PDGF) [12, 13, 28].

How can all these molecules mentioned above regulate osteoblastic proliferation and osteogenesis in osteoblastic metastases? Two key transcription factors, osterix and runt related-transcription factor 2 (RUNX2), have been recognised as downstream gene targets and mediate the skeletal preference of specific types of cancers [11, 13, 29, 31], resulting in increase of alkaline phosphatase and osteocalcin. Osterix (osx), a zinc finger-transcription factor, is essential for osteoblast's differentiation [29]. Overexpression of osx was associated with decreased osteolysis and inversely correlated with metastatic potential of cancer [13, 30]. A careful study by Cao et al. revealed that osterix was down-regulated in two mouse osteosarcoma cell lines. Reexpression of osx in the mouse osteosarcoma cells inhibited tumor cell growth *in vivo* and significantly reduced tumor incidence and lung metastasis following intratibial injection of transfected cells [29]. RUNX2 belongs to the mammalian Runt homology domain transcription factors and regulates osteoblastic architecture. Increased expression of RUNX2 was found in metastatic breast and prostate cancers [31]. Barnes et al. showed that one isoform of Runx2 regulates bone sialoprotein (bsp), a previously recognised skeletal metastasis-related protein in cooperation with Msx2, a known regulator of osteoblastic homeostasis of breast cancer cells, suggesting RUNX2 as an osteoblast-related transcription factor involved in metastatic process [32, 33]. Chemotactic cytokines (chemokines) are a family of small proteins and can induce directed cell migration (chemotaxis), via specific G-protein coupled receptors. The stromal cell-derived factor-1 (SDF-1 or CXCL12) and CXCR4 pathway has been implicated in the localization of prostate cancer to the bone marrow. High levels of SDF-1 were detected in the pelvis, tibia, femur, liver, and adrenal/kidneys. Blockade its receptor, CRCX4, with specific antibody could significantly diminish metastatic burden [34], supporting the function of this pathway to facilitate tumor seeding to bone [35].

The biological signature of osteolytic bone metastases has also been extensively studied. During this process a vicious circle necessary for the initiation and development of bone metastases is activated between tumor cells, osteoblasts, osteoclasts and bone matrix. The most studied tumor is breast cancer. Parathyroid hormone-related protein (PTHrP), increasingly produced by tumour cells, activates osteoblasts to release RANK ligand (RANKL) and osteopontenegrin (OPG) [36]. Osteoblasts and bone marrow stromal cells express OPG, which counteracts the RANKL-RANK (receptor activator of NF-kB) binding. This balance determines stimulation of osteoclasts [37] and leads to subsequent bone matrix degradation by matrix metaloproteinases (MMPs) and release of diverse growth factors such as DGF, GF-beta1, FGF and IGFs, resulting the restart of the circle by stimulating tumor cells to release PTHrP [36–38].

RANKL is a member of the TNF superfamily. RANKL is stimulated by PTHrP, TNF-α, IL-1, -6, -11 and PGE2 released from tumor cells. Binding with RANK, a transmembrane receptor of osteoclast precursors, leads to activation of RANKL that promote differentiation of pre-osteoclasts into mature, active osteoclasts [37]. A soluble receptor SRANK-Fc managed to reduce systemic bone remodelling markers including serum osteocalcin, bone-specific alkaline phosphatase and urine N-telopeptide of collagen and also reduced serum prostate-specific antigen levels and tumor volume in the bone with diminished overall progression of prostate tumor [39].

Osteoprotegerin (also called osteoclastogenesis inhibitory factor, OCIF), is a soluble decoy receptor found in osteoblasts and marrow stromal stem cells and inhibits osteoclastogenesis by interfering with the RANKL-RANK interaction [40]. Prostate carcinoma cells injected into the tibia of mice induced metastases formation, which was prevented by OPG. A significantly reduced number of osteoclasts at the periphery of the lesions was also observed [41]. Morony et al. demonstrated that OPG could prevent osteoclastogenesis and osteolytic lesions, induced by injection of human MDA-MB-231 breast cancer cells in nude mice [42].

Other important molecules which have shown a strong association with osteolytic metastases include CTGF (connective tissue growth factor), IL-11, activin A, follistatin (FST), MMP1, a disintegrin and metalloproteinase with thrombospondin motifs-1 (ADMATS1) and heparanase/proteoglycan [43–49].

Recent studies came to shed new light in the genetic signature of skeletal metastases. Kang et al. analysed the expression profiling of breast cancer cells with different metastatic potential to the bones, after serial transfection [50]. A series of 102 genes (43 overexpressed, 59 downexpressed) with altered expression was discovered in the highly metastatic group. Of them, four upregulated genes, CXCR4, CTGF (connective tissue growth factor), IL-11, and MMP1, were strongly correlated with metastatic dissemination. suggesting their involvement in certain biological processes including homing to the bone marrow, invasion, angiogenesis and osteoclastogenesis for cancer cells to metastasize to bone [50].

4 Lymph node metastases

The regional lymph node metastasis is a critical event in distal tumor spread and has an adverse predictive role for

most tumor types. During the last years, extensive gene expression studies have been made in order to identify specific molecular signatures for lymph node metastases [51]. In expression profiling of head and neck cancers using Affymetrix gene chip, O'Donnell et al. found a gene signature of totally 116 genes for prediction of lymph node metastasis. Of them, three genes, KCNJ5/GIRK4 and AP-Vacuolar ATP synthase subunit C2, have been previously associated with aggressive metastatic phenotype in breast and pancreas cancer. Characteristically, CXCR4, a well known chemokine receptor participating in hematogenous spread, did not show altered expression in lymph node metastases [52]. The extracapsular spread of lymph node metastasis is one of the most important negative prognostic factors of head and neck cancer [53]. Zhou and coworkers studied the expression profile in squamous cell carcinomas (SCC) of oral tongue with different metastatic ability using array technology and quantitative reverse transcriptase PCR [54]. Of the differentially expressed genes identified, six (BMP2, CTTN, EEF1A1, GTSE1, MMP9, and EGFR) were strongly associated with lymph node matastases, while five (BMP2, CTTN, EEF1A1, MMP9, and EGFR) were correlated to extracapsular lymph node spread. The authors emphasized the importance of combining different candidate gene lists generated by various methods in order to reduce mathematical biases and increase the possibility of identifying true molecular biomarkers with significant predictive power [54].

Roepman et al. investigated the genetic background of lymph node metastases deriving from primary SCC of the oral cavity and oropharynx. By using DNA microarrays, 103 genes with major predicting role were detected [55]. The same authors recognised that this expression signature is only a small subset of a larger group, consisted of totally 825 genes and suggested the significance of evaluating a high number of genes in order to increase the predictive power. From these, two gene subsets with increased expression are responsible for adhesion and degradation of the extracellular matrix, respectively. This paradox shows how tumor cells can invade surrounding tissues through a complex series of adhesion/deadhesion to the extracellular matrix (ECM) and highlight the diversity of metastatic cascade [56]. However, another study by the same group demonstrated that expression profile is extremely similar between primary and metastasic SCC of the head and neck. Only metastasis-associated gene 1 (MTA1) was differently expressed [57]. If lymph node metastases contain the same genetic information as the primary head and neck tumors and expression profile is not altered during malignant spread, then the metastatic and the primary tumor microenvironment must have many morphological and functional similarities. Further investigation is required to clarify this issue. The status of axillary lymph nodes in patients with breast carcinoma is one of the critical parameters for classification of tumor and treatment decisions. Zhu et al. detected 79 differentially expressed genes responsible for lymph node metastases in 26 breast cancers and found that several genes including MMP2, fibronectin, osteoblast specific factor 2, collagen type XI alpha 1 were downregulated in 30 lymph node metastases [59].

Prostate cancer often migrates through the lymphatic route, depositing tumor cells into pelvic lymph nodes. Orthotopic implantation of PC-3 prostatic carcinoma cells into mice resulted in metastases in paraaortic lymph nodes. An array analysis revealed that genes responsible for matrix degradation (cathepsins, MMPs, plasminogen activator urokinase receptor), cell adhesion (integrins) and transcription (Ets, ETV) presented significantly increased expression in the metastatic prostate carcinomas [60]. Similarly, the involvement of locoregional lymph node is one of the most important prognostic factors in lung cancer. Koyabashi et al. identified a gene signature of 40 genes being responsible for lymph node metastasis in 18 human lung adenocarcinomas by using array assay and laser-capture microdissection (LCM) [61].

5 Brain metastases

Brain metastases are the most common type of intracranial tumor occurring mainly in patients with breast, lung and colorectal cancer as well as melanoma. Approximately, 20–30% of patients with primary tumors develop metastases to the brain [62]. Angiogenesis is necessary for growth and development of tumors. Clinical and experimental evidence has suggested that spreading of tumor cells and formation of metastases are directly related to the number of microvessels in the primary tumor. Brain metastases selected from the breast cancer variants MDA231-BR1, -BR2 and -BR3 with increased metastatic ability were characterised by richer vascularity in comparison to variants with lower spreading potential. Treatment with VEGF-A inhibitor PTK787/Z 222584 resulted in shrinkage of brain metastatic lesions and diminished vascularisation, implicating VEGF as a contributor of brain metastases of primary breast cancer [63]. However, a previous study has shown that major VEGF homologues such as VEGF121 and VEGF125 are necessary but not sufficient for formation of brain metastases [64].

Astrocytes produce various cytokines and growth factors such as IL1, IL-6, TNG-alpha, TGF-β and, IFN-gamma and PDGF and modulate in this way brain microenvironment [65]. MDA-MB-435 BR1 breast cancer cells derived from MDA-MB-435-induced brain metastases showed increased adhesion to astrocytes, which was reversed by antibodies against IL-6, TGF-β and IGF-1. Therefore, glial

cells may, through release of these chemokines and growth factors, favour specific breast cancer seeding to brain parenchyma [65]. Additionally, various signal neurotransmitters secreted by brain cells can influence development of metastases and seeding into the central nervous system [66]. Drell et al. provided an elegant insight by combining video microscopy and computer-assisted cell tracking to study the spreading behaviour of breast carcinoma cells in response to various neurotransmitters. In this report, metenkephalin, substance P, bombesin, dopamine, and norepinephrine enhanced while gamma-aminobutyric acid (GABA) inhibited chemotactic cell migration [67].

The discovery of silence of tumor-related genes in metastatic cascade came to shed new light in the complex nature of tumor colonization. The metastasis suppressor genes (MSGs) suppress the formation of spontaneous, macroscopic metastases without affecting the growth rate of the primary tumor [68]. To date, several MSGs have been identified such as Nm23, differentiation related gene (Drg-1), Src-suppressed C Kinase substrate (SSeCKs), Vitamin D3 upregulated protein 1 (VDUP), CRSP3 transcriptional coactivator, mitogen activated protein kinase kinase 4 (MKK4), Raf kinase inhibitor Rkip, RhoGDI2, Brms1, Kiss-1, Claudin-4, and Kai [68, 69]. MSGs do not affect common tumor characteristics such as cell proliferation, invasion and angiogenesis. In contrast, their role is probably focused during the late stages of tumor cell spread and colonization [68]. Stark et al. studied the expression of important MSGs like KISS1, KAI1, BRMS1, and Mkk4 in brain metastases from ductal invasive breast cancer. Prominent reduction in transcriptional and protein expression of these MSGs was detected in comparison to the primary malignant lesions [70].

Brain metastases from malignant melanoma have a poor prognosis characterised by rapid disease progression. Fidler et al. investigated the seed and soil hypothesis by injecting melanoma cells into both internal and external carotid arteries of mice. B16v melanoma cells colonized exclusively cerebral ventricles and meninges while K-1735 melanoma cells seeded specifically into brain parenchyma, independently of their injection site [71, 72].

Boukerche et al. analysed the role of Mr 55,000 membrane protein in different melanoma variants. MR 55,000 was directly correlated to progressed and metastatic melanomas while no expression was found in melanocytes, nevi, or radial growth phase primary melanomas, indicating this protein as a new metastatic marker [73].

Accumulating evidence suggests that the signal transducer and activator of transcription 3 (Stat3) plays a critical role in development of metastases [74]. Recent studies revealed higher Stat3 activity and expression in melanoma brain metastases in comparison to primary melanomas. In addition, overexpression of Stat3 strengthened melanoma angiogenetic and invasive potential and promoted brain metastases, suggesting Stat3 as a new target for preventing and treating melanoma metastases to the brain [74, 75].

The reexpression of metastasis suppressor gene KiSS-1 could be detected only in melanoma cells with inhibited metastatic ability by transfer of human chromosome 6. Melanoma cells transfected with KiSS-1 showed significant decrease in metastatic ability [76]. Melanomas with low nm23 expression were highly predisposed to develop metastases to the brain, suggesting the predictive role of NM23 in tumor metastasis [77].

During the last years, several studies have reported the role of neutotrophins in melanoma brain metastases. Malignant melanoma cells express the cell-surface receptor $p75^{NTR}$ that binds to its ligand, nerve growth factor (NGF), produced by brain parenchymal cells such as astrocytes [78]. Neutrotrophins can increase malignant invasion by increasing secretion of heparanase, an extracellular matrix (ECM)-degrading enzyme that degrades heparan sulfate proteoglycans [79]. Marchetti et al. have shown that astrocytes participate, through increase in heparanase activity, in brain-specific colonization by malignant melanoma cells, revealing a new interaction mechanism between melanoma and normal glial cells [80].

The receptor tyrosine kinase HER-2/neu correlates with more aggressive disease, increased metastatic potential to the brain, and a poorer prognosis in patients with breast cancer [81]. Targeted therapy with Her-2/neu antibody presented survival benefit for breast cancer patients with brain metastases [82]. A subset of these patients still developed brain metastases, most probably due to poor trastuzumab (anti-Her-2/neu antibody) permeability across the blood brain barrier (BBB), prompting the development of new Her-2/neu inhibitors to overcome this problem [81, 82]. However, in a recent study, Palmieri et al. demonstrated that both Her-2-transfected and control cells produced similar numbers of brain micrometastases in injected athymic mice, except difference in tumor volume [83].

6 Lung metastases

The KiSS-1 on chromosome 1q32-q41 encodes metastin, an endogenous ligand of the orphan G-protein-coupled receptor hOT7T175. Expression of metastin significantly decreased pulmonary metastases of B16-BL6 melanoma cells transfected with hOT7T175 [84]. In another work, Lee et al. transfected Kiss-1 in MDA-MB-435 breast cancer cells and injected them into the mammary fat pads of athymic mice. Kiss-1 transfectants significantly reduced the number of pulmonary metastases through cell adhesion mechanisms without affecting tumorigenic ability [85]. These suggested Kiss-1/metastin as a new therapeutic opportunity for pre-

vention and treatment of melanoma-induced pulmonary metastases.

The DARC (Duffy antigen/receptor for chemokines) encodes an endothelial cell surface protein and has been identified as essential binding protein for KAI1. The interaction between DARC and KAI1 transmitted a TBX2-/p21-mediated senescent signal to tumor cells, inhibiting their proliferation. In DARC-knockout mice, melanoma cells expressing KAI1 showed reducing metastatic ability, implicated the metastasis-suppression activity of KAI1, and the potential therapeutic usage of this pathway for lung metastases [86].

Amplification and overexpression of ACK1 (activated Cdc42-associated kinase 1) was closely associated with enhanced cellular motility, invasiveness and metastases to the lungs of breast cancer cell lines via enhanced activation of p130Cas and Rac pathway [87]. In line to previous genetic analysis of metastatic sufficiency, ACK1 did not affect the tumorigenic potential of primary breast cancer cells [87].

The gene expression study on breast cancer cell lines with different metastatic ability has identified 171 genes closely associated with pulmonary metastases including many GTPases. Stable transfection of the deleted in liver cancer-1 (DLC-1) GTPase in metastatic M4A4 cells decreased metastatic spread into the lungs of athymic mice, underlying metastasis-suppression role for DLC-1 [88].

Zhang et al. studied the expression profile of endothelial cell-associated genes in hepatocellular carcinomas (HCC) and noted overexpression of PDGFR-α only in the endothelium of highly metastatic HCC. Furthermore, inhibition of PDGFR using the receptor tyrosine kinase inhibitor imatinib mesylate (Gleevec) suppressed metastases of HCC to the lungs, suggesting PDGFR-α as a specific genetic marker of pulmonary metastases of HCC [89].

Brown and Rhuoslahti detected increased expression of metadherin protein in breast cancer and found that metadhern binds to pulmonary vessels using C-terminal segment in the extracellular domain. Both intravenous and intracardiac injection of 4T1 breast cancer cells incorporating metadherin phage revealed specific seeding to pulmonary vasculature without adhering to other sites such as bone, brain or liver. Blockade of metadherin activity resulted in suppression of pulmonary metastases [90].

Ezrin, a protein that plays important role in cytoskeletal organization, was overexpressed in early metastatic lesions and mediated metastatic survival of osteosarcoma to the lungs [91].

A new set of genes consisted of 95 genes that was clinically associated with the development of pulmonary metastases of breast cancer, was discovered. This new signature was separated into two groups [92]. The first one, metastagenicity genes, included genes with an important role in the modulation of primary tumor microenvironment such as growth factors (the HER/ErbB receptor ligand epiregulin), chemokines (CXCL1), cell adhesion receptors (ROBO1), extracellular proteases (MMP1), intracellular enzymes (COX2) and transcriptional regulators (ID1). The second subset included genes such as secreted protein acidic and rich in cysteine (SPARC), MMP2 and vascular cell adhesion molecule (VCAM) that were overexpressed only in virulently metastatic cells without affecting primary tumor growth and included known extracellular proteins. The first group of genes contributed to the metastatic process while virulence genes mediated lung-specific seeding of breast cancer cells. This work presented by Minn et al. provided direct evidence regarding the molecular identity of site-specific metastasis [92].

7 Liver metastases

Liver metastases represent a major cause of death for patients with colorectal and pancreatic cancer and hepatocellular carcinoma. With the advent of gene expression microarrays and the establishment of improved study models, several new factors have been added in the list of gene signature responsible for hepatic metastases.

Two key angiogenetic players, PDGFR-alpha and beta, were found to be expressed in pancreatic cells and tumor-associated endothelial cells. PDGFR-beta was activated both in pancreatic tumor and liver metastases. Inhibition of PDGFR with specific monoclonal antibody in combination with gemcitabine reduced tumor growth and inhibited spontaneous liver metastasis, underscoring PDGFR significance in pancreatic cancer progress and metastasis [93, 94].

KAI1/CD82, a member of the transmembrane 4 superfamily (tetraspanin), has been previously shown to contribute to metastagenicity of malignant melanoma [69, 84]. A splice variant of KAI1 lacking exon 7 at the C-terminal region interacts with KITENIN, a tetraspanin family member found to be overexpressed in metastatic gastric tumors. Transfection of CT-26 colorectal cancer cells with this variant facilitated cytoskeletal reorganization and resulted in early liver metastases. In this study, KITENIN was closely associated with suppression of KAI1 and two other well known MSGs, nm23 and KiSS1, suggesting KITENIN targeting to fight metastasis [95].

The tyrosine phosphatise PRL-3, has been implicated as important marker for liver metastases [96]. Increased PRL-3 expression was found in metastatic colorectal carcinomas and downregulation of PRL-3 in DLD-1 colon cancer cells prevented hepatic metastases without affecting cell proliferation while its transfection increased metachronous liver and lung metastases [97, 98]. SW480 colorectal cancer cells overexpressing PRL-1 and PRL-3 presented upregulation of RhoA and RhoC GTPases and inhibition of Rho kinase

activity abrogated their cell motility. In addition, farnesylation of PRL-3 and preservation of its phosphatase activity were essential for invasion of tumor cells [99]. Recently, specific monoclonal antibodies against PRL-3 were developed for predicting and diagnosing colorectal cancer hepatic metastasis [100].

Miyamoto et al. investigated the role of insulin-like growth factors I and II (IGFI/II) in colorectal cancer. Blockade of IGFI/II using neutralising antibodies strongly diminished formation of hepatic metastases from colorectal cancer cells injected intrasplenically [101]. Inhibition of VEGF has offer new opportunities in advanced/metastatic pancreatic and colorectal cancer to the liver. But additional studies are warranted to optimize therapeutic targeting of angiogenetic mechanisms for treating liver metastases [102–105].

Inhibition of Src tyrosine kinase in combination with gemcitabine suppressed metastases to the liver and locoregional lymph nodes [106]. Furthermore, rapamycin in combination with antiangiogenetic factors possessed antimetastatic activity in another orthotopic pancreatic cancer model [107].

In contrast to most other tissue malignancies, HCC has as a primary metastatic target the liver itself [108]. Ye et al. studied the gene expression of intra-hepatic metastatic HCC from patients previously infected with hepatitis-B virus (HBV), using tissue microarrays. Strikingly, the genetic signature was similar between primary and metastatic HCC and no gene was found as predictive marker for distinguishing primary from metastatic lesions. This is consistent with previous reports that primary tumors are genetically programmed to metastasize at a very early stage [109]. However, other studies identified genetic signature of HCC including several factors with an important role in cell adhesion and matrix degradation such as IL-2R, MMP-9, CD37, a9-integrin, serine protease member-5 and osteopontin. Osteopontin was strongly upregulated in metastatic samples and its blockade resulted in inhibition of HCC cells migration and metastatic spread to lungs [110].

A recent report detected a decrease in pro-inflammatory Th1-like cytokines and a major increase in the production of anti-inflammatory Th2-like cytokines in livers bearing metastatic HCC while no changes in their status were found in unaffected hepatic tissue. In this study, Budhu et al. identified expression signature including 17 genes, for prediction of venous HCC metastases and suggested that liver microenvironment might, through alteration of its immune response, favour metastatic spread of HCC [111].

8 Conclusions

More than a century since Paget noticed that breast tumors were metastasizing to the liver, the seed and soil theory gains identity. Numerous factors have already been found and probably many more will be discovered that determine specific interaction between tumor cells and organ microenvironment and favour in this way metastatic spread to preferential body sites (Fig. 1). The discovery of metastatic

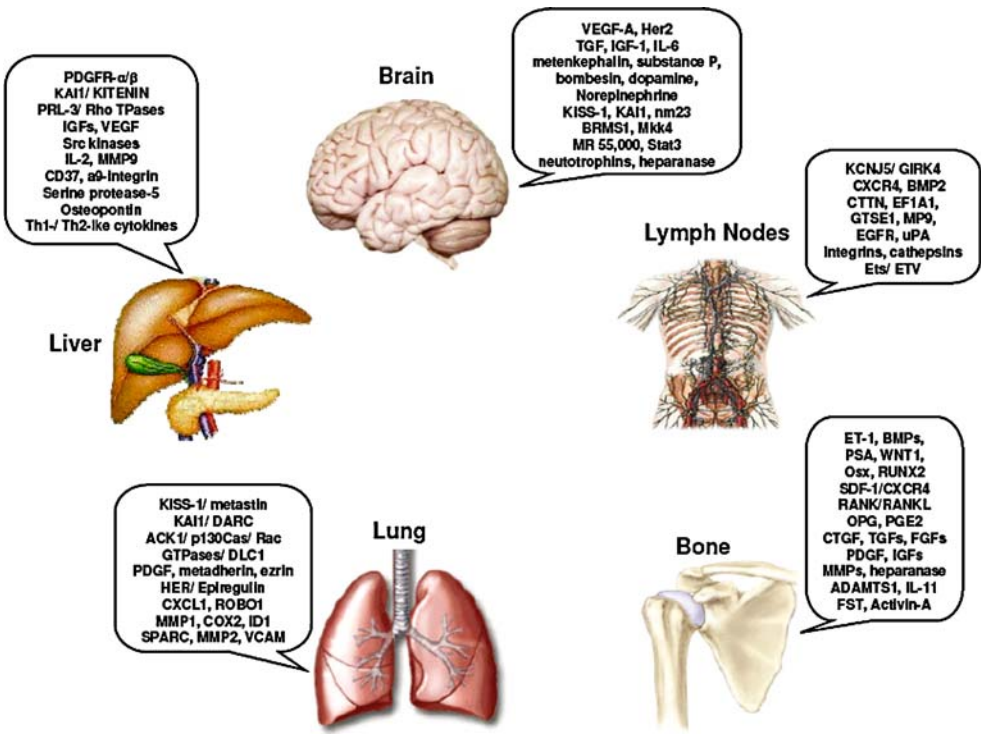

Fig. 1 Summary of factors influencing organ-specific metastases to the liver, lung, brain, bone and lymph nodes

gene signatures has undoubtedly shed new light in the pathophysiology of metastases. Microarray studies have led to the discovery of long lists of genes correlated with metastatic development. But at the same time, the complexity and discrepancy of huge amount of new data available today have raised major questions. How should we choose critical genes in order to prevent tumor spread and optimize antimetastatic therapy? The precise characterization and selection from these long lists of the genes that will offer to patients with cancer a true clinical benefit is the first obstacle to be overcome.

Another raised question is how the current therapeutic modalities affect all the genes/ pathways responsible for metastases. Despite the major advances in understanding tumor biology, today's cancer treatment is inefficient in a large amount of patients, who finally develop metastases to various organs. Will it be thoughtful to investigate whether the current therapeutic modalities affect metastasis-associated genes such as metastasis suppressor genes? Studies which incorporate the treatment of choice for each tumor as it is used in daily clinical practice today are desperately missing and it will be interesting to see if conventional chemotherapy or radiotherapy modifies the expression profile of the metastatic gene signature.

The third question that will have to be answered regards the optimal time point for genetically-based intervention for prevention and/or treatment of metastases. Today, most novel biological treatment agents are tested on patients with already established metastases and fail to show any significant clinical benefit. This is mainly due to the present lack of imaging and diagnostic methods sensitive enough to detect metastasis at its very beginning. The development of new antimetastatic compounds validated to act at the earliest possible stage of metastatic spread might prove to be more efficient in the future.

The advent of new technologies and the establishment of better tumor study models have led to the discovery of novel gene signatures which determine metastatic spread to specific body sites. Surprisingly, several studies have revealed a similar expression profile between primary tumors and their metastatic lesions. This new finding is of paramount importance and suggests early analysis of primary tumors expression profile with the purpose of identifying key factors for therapeutic management of metastases. Future, genetic signature-based, clinical studies should be designed with the purpose of classifying, at the earliest possible stage, patients with cancer who are likely to develop distant metastases. The latter will determine which patients will be benefited from therapeutic targeting of metastatic-responsible genes and pathways and at the same time will prevent unnecessary treatment, rendering metastatic treatment cost-effective.

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