

Review

Molecular Basis of Lung Tropism of Metastasis

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Abstract. *A predilection of metastasis to the lungs has been noted for several types of cancer. Herein, we summarize underlying mechanisms for lung tropism of metastasis. We discuss the identification of a gene signature in primary breast tumors predicting metastasis to the lungs, as well as functional validation of selected genes of the signature. We outline the contribution of pre- and metastatic niches, the role of exosomes, activation of disseminated, dormant tumor cells and selected tumor–stromal cell interactions to lung metastasis and colonization. We also refer to metastasis-mediating mechanisms based on alterations of the tumor cell cytoskeleton, as well as lung metastasis-suppressing mechanisms.*

Metastasis involves distinct steps, such as development of an aggressive tumor phenotype, intravasation, survival of tumor cells in transit, interaction with distant accomplices, extravasation, homing, formation of micrometastases, co-option of stroma in distant organs and finally their full colonization (1). The 'seed-and soil' hypothesis has postulated preferential colonization of pre-metastatic niches in distant organs (the 'soil') by circulating tumor cells (CTCs) derived from different tumor types (the 'seed') based on optimal interactions between tumor cells and the corresponding microenvironment (1, 2). Now, it has been confirmed that several types of tumors, such as breast cancer, melanoma, sarcoma and kidney cancer have a predilection for metastasis to the lungs. Other types of tumors such as colon, bladder, head-and-neck and pancreatic cancers, albeit not preferentially, also metastasize to the lungs (3,4). Early pre-clinical experiments in mice demonstrated preferential metastasis of lung-homing melanoma cells (5). After

implantation of small tissue fragments derived from different organs, it was found that melanoma cells only metastasize to normal lung and ectopically placed lung tissue (5).

In order to metastasize, some of the cells that disseminate from the tumor eventually reach the bloodstream. During circulation the tumor cells reach the lung where they come into contact with as much as 100 m² of vascular surface (6, 7). Tumor cells have a diameter five-times larger than that of pulmonary capillaries, which leads to them becoming stuck there and promotes tumor cell extravasation into the lung (1, 8). The lung capillaries are lined with endothelial cells that are surrounded by a basement membrane and adjacent alveolar cells. Traversing the basement membrane by CTCs requires expression of specific mediators of transendothelial migration. These anatomical constellations, in addition to genetically-based interactions discussed in more detail in the following text, contribute to the fact that the lungs are the second most common site for metastasis to occur. Targets discussed in this review, as well as molecular interactions between tumor cells, fibroblasts, macrophages and endothelial cells, are shown in Figure 1.

Niche

The concept that the formation and development of metastases is dependent on the formation of pre- and metastatic niches was solidified by experimental evidence (9-12). Generic as well as target organ-specific mediators are probably involved in niche formation. Potential pre- and metastatic niches in the lung are located around terminal bronchioles and bronchiolar veins (13). The concept of the pre-metastatic niche in the lung has been substantiated by studies with Lewis lung carcinoma (LLC) and B16 melanoma tumor-bearing mice. The lungs of these mice showed an increased infiltration of myeloid cells expressing macrophage antigen 1 (MAC1, CD11b, integrin α M) compared to non tumor-bearing mice. Vascular endothelial growth factor-A (VEGFA), transforming growth factor- β (TGF β) and tumor necrosis factor- α (TNF α) secreted by the

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Path Designer LungCancerPathway

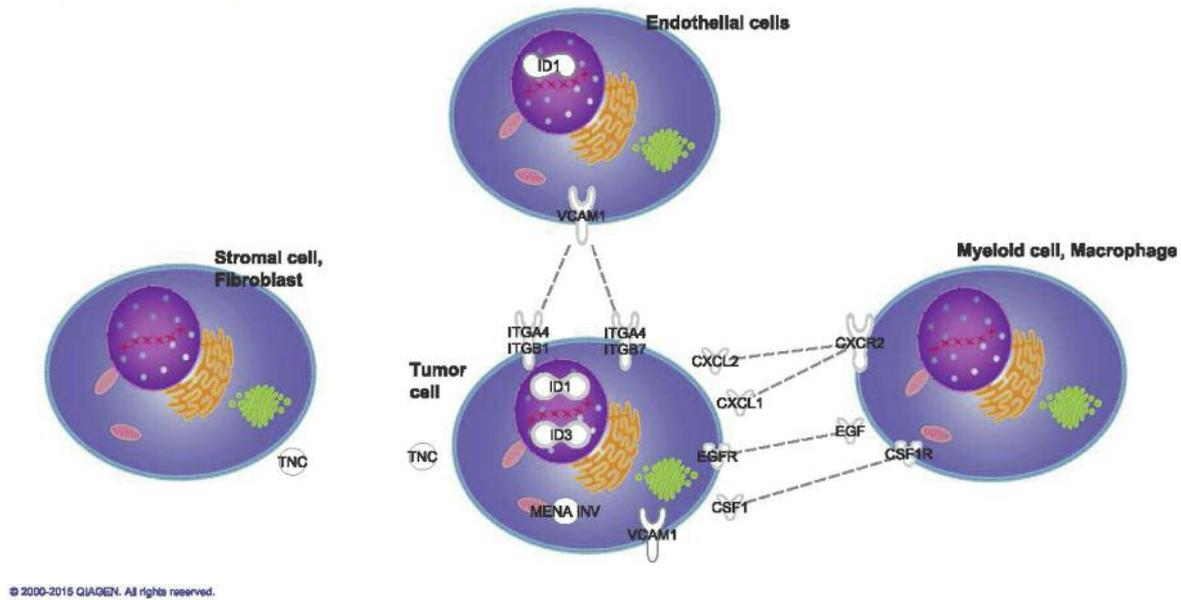


Figure 1. Schematic overview of targets discussed in this review. Molecular interactions between tumor cells and endothelial cells, stromal cells (such as fibroblasts) and myeloid cells (such as macrophages) are shown. Ingenuity pathway analysis was used for designing the figure. CSF1: Colony-stimulating factor 1; CSF1R: colony-stimulating factor 1 receptor; CXCL1,2: C-X-C motif ligand 1,2; CXCR2: C-X-C chemokine receptor 2; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; ID1/3: helix-loop-helix transcription factor modulator ID1/3; INTA4: integrin $\alpha 4$; INTB1,7: integrin $\beta 1,7$; MENA INV: invasive splice variant of MENA; TNC: tenascin C; VCAM1: vascular cell adhesion molecule 1.

primary tumor activates lung endothelial cells to express and release S100 calcium-binding proteins A8 and A9 (S100A8 and S100A9). The inflammatory chemoattractants recruit MAC1⁺ myeloid cells to the pre-metastatic niche of the lung, but not to other organs such as kidney and liver (14-16). S100A8 also acts as a chemoattractant for tumor cells. Activation of mitogen-activated protein kinase p38 (p38MAPK) signaling was a prerequisite for recruitment of both cell types. Lung colonization and recruitment of MAC1⁺ myeloid cells was inhibited by 90% using monoclonal antibodies directed against S100A8 and S100A9, pinpointing similar mechanisms involved in recruitment of these cells. There is evidence that the recruitment of myeloid and tumor cells is mediated by migration-stimulatory factors with specificity for the corresponding distant organ of the pre-metastatic site. The factor responsible for inducing S100A8 and S100A9 was identified as serum amyloid A3 through interaction with toll-like receptor 4 (TLR4) on macrophages and tumor cells (17-19). Further evidence for the importance of the interplay between tumor cells and stromal cells for niche formation and lung colonization was obtained with an LLC-carcinoma-based tail vein metastasis model (20). Lung colonization was strictly dependent on the interaction between tumor cell-secreted versican and TLR2 on host macrophages, resulting in secretion of TNF α .

Recruitment of bone marrow-derived hematopoietic progenitor cells is a subsequent step in the evolution of a metastatic niche in the lungs. Modification of the extracellular matrix (ECM) by fibronectin and lysyl-oxidase secretion from activated fibroblasts facilitates engraftment of metastatic tumor cells to the niche for colonization with micrometastases (21). Finally, progression of the early metastatic niche to a niche supporting the progression of micrometastases to macrometastases is mediated by an angiogenic switch due to recruitment of endothelial progenitor cells (5, 22).

Exosomes

Exosomes have been shown to be involved in metastasis and creation of metastatic niches. Exosomes are small vesicles derived from the endocytic pathway and are abundantly secreted by tumor cells. They carry genomic and proteomic signatures characteristic of the tumor they are derived from. Exosomes have been associated with many steps of tumor pathogenesis (23-26). Most recently, a role in microRNA biogenesis has been associated with promotion of tumorigenesis (27). Additionally, exosome transfer from stromal cells to breast cancer cells, resulting in regulation of therapy resistance pathways, has been demonstrated (28).

In a rat model of pancreatic adenocarcinoma, it was shown that exosomes can act over long distances and create a metastatic environment for tumor cells which is dependent on tumor cell-expressed cluster of differentiation 44 variant 6 (CD44v6) (29). Pulmonary vascular destabilization is an early step facilitating lung metastasis (30). The role of exosomes in enhancing lung permeability in mice was shown with exosomes derived from highly (B16F10) and poorly (B16F0) metastatic melanoma cells after tail vein injection into mice by monitoring extravasated dextran (31). Only exosomes derived from the B16F10 cell line were able to enhance lung permeability. A 240-fold increase in tumor burden in the lungs was noted when exosomes derived from B16F10 cells were intravenously injected prior to implantation of B16F0 melanoma cells (31). Transcriptional profiling of lung tissue after injection of exosomes derived from B16F10 cells revealed 130 differentially expressed genes. Among the genes identified were genes involved in ECM remodeling and inflammation, heat-shock proteins and effectors of metastatic niche formation such as S100A8 and S100A9.

Dormancy

Disseminated tumor cells which have penetrated into the lung parenchyma can undergo dormancy because they are not adapted to the new microenvironment (32-34). At this stage, cell division and apoptosis occur at similar rates; therefore, micrometastatic lesions are not expanded. Micrometastases contain only a small number of dividing cells and are therefore resistant to antimetabolic agents (35). These complex processes can be recapitulated in mouse models (36-39). B16 melanoma cells disseminated to the lungs can undergo a protracted state of senescence (36). The dormant state is due to lack of signaling by ECM components such as integrins and urokinase-type plasminogen activator receptor (uPAR) (8). An inhibitory effect on tumor cells is exerted by bone morphogenetic proteins (BMPs) derived from stromal cells acting as antagonists of wingless-type MMTV integration site family member (WNT) signaling (39). A number of pathways have been shown to activate dormant tumor cells in the lungs. One mechanism is based on activation of extracellular signal-regulated kinases (ERK) and downregulation of mitogen-activated protein kinase p38 (40-42). For example, interaction of fibronectin (FN) with the uPAR- $\alpha 5\beta 1$ complex was shown to activate ERK signaling and to down-regulate p38 (45). Additionally, DAN domain family member 5 (DAND5), an inhibitor of lung-derived BMP ligands, is able to activate dormant cancer cells in the lungs, resulting in outgrowth of metastases (40). A DAND5-linked tumor gene signature predicts metastatic relapse to the lungs (40). Finally, tenascin secreted by disseminated tumor cells or

stromal cells can stimulate WNT and NOTCH signaling (41-43). Stroma-derived periostin can also recruit WNT ligands, thereby augmenting WNT signaling (44). Interaction of fibronectin with the uPAR- $\alpha 5\beta 1$ complex was shown to activate ERK signaling and to down-regulate p38 (45). Activating interactions probably occur in the context of a metastatic niche (32) as described earlier.

Cytoskeleton

Alterations of the cytoskeleton have an impact on cell adhesion, migration and metastasis. MENA, one of the three members of the Ena/Vasp homology proteins, is a cytoplasmic protein which plays a role in extension of actin fibers and therefore acts as a cytoskeleton remodeling protein. Extension of actin fibers is normally inhibited by a capping protein which is removed by cleavage of the ends of the fibers by cofilin, permitting their elongation. During epithelial mesenchymal transition, a MENA splice variant (MENA INV), which contains exon 16, is expressed in tumor cells (46). This variant is especially active in promoting fiber elongation and growth of lamellopodia and filopodia, as well as acting as a mediator of epidermal growth factor (EGF)-induced cell motility and transendothelial invasiveness (47, 48). In xenograft models, carcinoma cells transfected with this variant exhibited a six-fold increase in lung metastasis. Possible involvement of MENA INV in metastasis to the lungs of defined types of human tumors needs to be investigated in more detail. Analysis of steady-state levels of RNA for MENA INV in comparison to MENA in tumor tissues *versus* corresponding normal tissues based on The Cancer Genome Atlas (TCGA) revealed no change of MENA INV RNA in invasive breast cancer, significant increase of MENA INV RNA in prostate adenocarcinoma and an inverse relationship in renal cell carcinoma (RCC) as shown in Figure 2. For further analysis, data correlating lung metastasis with MENA INV RNA should be generated in sub-groups of patients with known status of lung metastasis.

RhoC, a member of the Rho-family of small (21 kDa) Ca-dependent GTPases, functions as a regulator of the actin-based cytoskeleton and has been implicated in metastasis of cancer to the lungs (49, 50). This was strongly supported in a breast cancer metastasis model (PY-MT) that was deficient for RhoC. Although the tumors formed normally, there was almost no lung metastasis and impaired cell motility was observed in cells derived from the mammary tumors (49). Microarray profiling of selected melanoma cell lines has identified RhoC as a mediator of lung metastasis in mice (50). RhoC enhances metastasis when overexpressed, dominant negative RhoC inhibits metastasis. RhoC also increases the abundance and metastatic potential of breast cancer stem cells (51); is overexpressed in 90% of inflammatory breast carcinoma, the most lethal form of

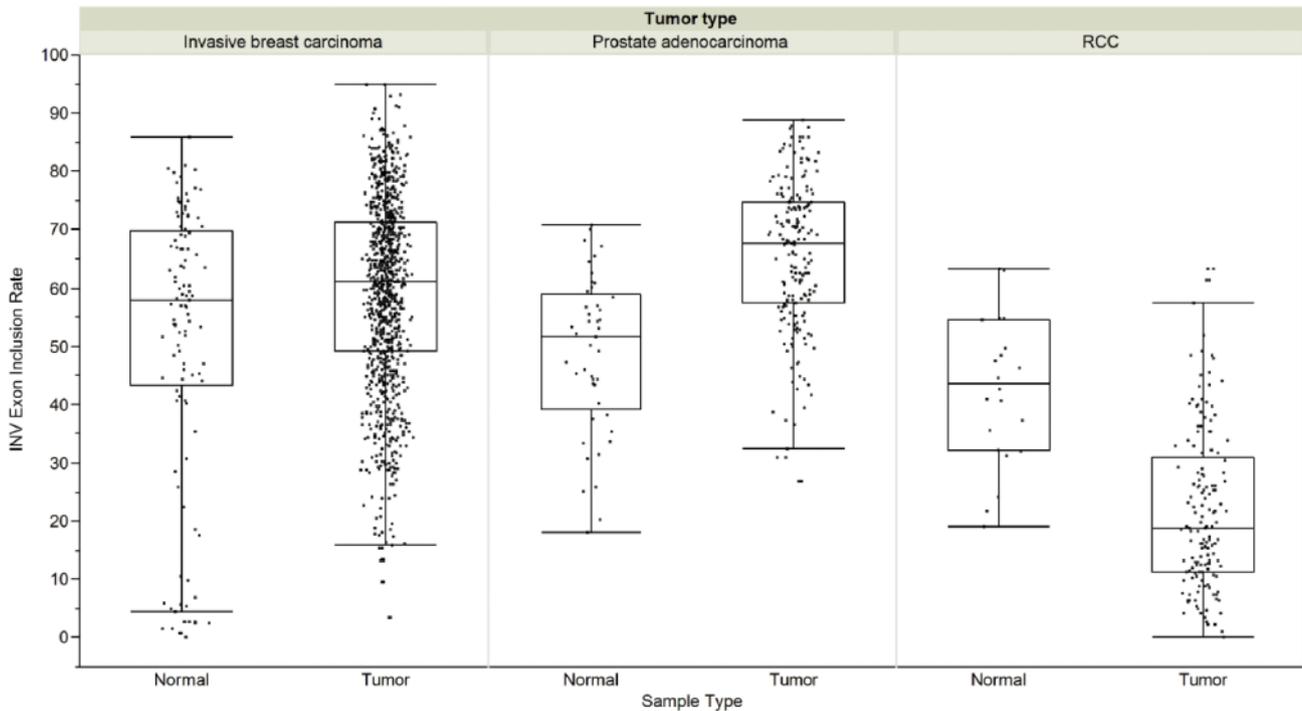


Figure 2. Inclusion rate of MENA exon 11 in transcripts from The Cancer Genome Atlas (TCGA). Cohorts for invasive breast carcinoma: 111 matched normal, 1046 tumor samples; prostate adenocarcinoma: 51 matched normal, 360 tumor samples; and renal clear cell carcinoma (RCC): 72 matched normal, 519 tumor samples. Expression was measured by whole-transcriptome sequencing and splice junction counts as derived from the Broad FIREHOSE portal were used to compute inclusion rates. The percentage of inclusion rate of exon 11 in MENA transcripts is shown and represents the percentage of reads indicating inclusion of exon 11 versus the percentage indicating a skipping event of exon 11. Boxes contain 50% of the samples within the box, while the whiskers include all other samples except for outliers. The inclusion rate was measured based on RNASeq splice junction data indicating inclusion of the exon versus splice junction counts indicating an exon skipping event. Normal tissue represents matched adjacent normal tissue of patients with cancer.

breast cancer (51); and has been implicated in metastasis of head-and-neck cancer (52).

Integrin β 1/focal adhesion kinase (FAK) signaling has been shown to mediate tumor cell invasion and proliferation of cancer cells disseminated to the lungs by multiple pathways, including cytoskeletal signaling (53-55). From a mechanistic point of view, integrin β 1 was shown to activate a dual kinase complex consisting of FAK and rouse sarcoma cellular homolog (SRC). This complex is able to bind and phosphorylate various adaptor proteins such as p130 crk-associated substrate (CAS) and paxillin, and is activated in many tumor cells, generating signals leading to tumor growth and metastasis (53-55). Neural precursor cell expressed, developmentally down-regulated gene 9 (NEDD9), an adaptor protein which enhances focal contact formation and invasion, is amplified in an H-Ras-driven mouse melanoma model and in metastatic human melanomas (56). Ezrin, a cytoplasmic peripheral membrane protein acting as a cytoskeletal organizer has been identified as an important mediator of metastasis of pediatric sarcomas to the lungs (57,

58). In a pediatric rhabdomyosarcoma model, increased expression of the homeobox transcription factor six homeobox 1 (SIX1) was observed, which acted as an inducer of ezrin in lung metastatic variants (57). Up-regulation of ezrin has also been noted in metastatic osteosarcoma (58), underlining selective pressure for ezrin expression in pediatric sarcoma metastasis.

Lung Metastasis Modifier Genes

Patients with estrogen receptor-negative breast cancer very frequently experience relapse with lung metastasis. Retinoic acid receptor responder 3 (RARRES3) was identified as a suppressor of breast cancer metastasis to lung based on regulation of adhesion to lung parenchyma and differentiation (59). In MDA-MB-231-LM2 breast cancer cells, RARRES3 prevents metastasis to the lungs (59). Reduced expression of RARRES3 in a sub-group of patients with breast cancer allows identification of those who are more likely to develop lung metastasis (59). RARRES3

exhibits phospholipase A1/2 catalytic activity which stimulates proliferation, whereas the competence for adhesion to the lung parenchyma is mediated by a domain independent of the enzymatic activity. RARRES3 can be induced by retinoic acid. Therefore, use of retinoic acid in the adjuvant setting might be an option to induce RARRES3-based metastasis-suppressive function.

Signal-induced proliferation-associated gene 1 (SIPA) was identified as a candidate modifier of metastasis to the lungs (60). SIPA1 is a GTPase activating protein that modulates the activity of RAS-related protein (RAP) 1. SIPA1 overexpression increases metastatic capacity as shown with spontaneous metastasis assays of transfectants, and overexpression of SIPA1 is associated with metastatic progression of several human cancer types (60). Interestingly, a polymorphism in SIPA1 due to a nonsynonymous amino acid substitution hindering the RAP-GTPase function attenuates pulmonary metastasis (60).

ECM and Lung Metastasis

The ECM plays a key role for metastasis of breast cancer to the lungs in early stages of metastasis and in the context of the metastatic niche (61). Herein we highlight the role of tenascin C and periostin in metastasis of breast cancer cells to the lungs. A correlation between expression of tenascin C and breast cancer metastasis has been reported (62). Periostin knock-out mice develop polyoma middle T antigen (PY-MT)-driven mammary tumors, however, lung metastasis is significantly diminished compared to PY-MT-driven tumors in wild-type mice (63). A bi-phasic role for tenascin C in lung colonization of breast cancer cells has been identified (62). Ablation of tenascin C in disseminated cells early in the metastatic process inhibits the outgrowth of lung metastases, whereas late inhibition does not affect progression from micro- to macrometastasis. This indicates the essential role of cancer cell-derived tenascin C for metastatic outgrowth until the tumor stroma takes over as a source for tenascin C, emphasizing the role of tenascin C as a 'seed' and 'soil-intrinsic' prometastatic factor. Periostin and tenascin C can enhance WNT and NOTCH signaling support for metastasis-initiating cells by activating developmental pathways increasing the viability of these cells. The role of the microenvironment is underlined by the fact periostin as well as TNF α can be produced by transforming growth factor β 3 (TGF β 3)-stimulated myofibroblasts (63). Periostin can bind to stromal WNT and present WNT to stem-like, metastasis-initiating cells and is also able to bind to tenascin C, anchoring it to ECM components such as fibronectin and type I collagen. Tenascin C can also increase the concentration of growth factors such as EGF and fibroblast growth factor and interact with fibronectin, heparin-sulfate proteoglycans, fibrinogen, integrins, matrix metalloproteinases (MMPs) and EGF receptor (EGFR). Tenascin C was shown to activate

NOTCH signaling by means of musashi, an RNA-binding protein, which acts through repression of translation of the NOTCH inhibitor mNumb (64). Furthermore, tenascin C mediates induction of leucine-repeat containing G protein-coupled receptor 5 (LRG5), a target of the WNT pathway and a marker of stem cells (65).

Identification of a Gene-based Signature Mediating Metastasis to the Lungs

Several classes of genes involved in tumorigenesis and metastasis have been identified (3, 4, 66) which play a role in tumor initiation, tumor progression, metastasis initiation, metastatic progression and metastatic virulence. Genes involved in metastatic virulence confer a selective advantage on tumor cells in distant organs, but not for the pathogenesis of the primary tumor. Virulence genes can promote intra- and extravasation, survival in the circulation, adaptation to survival and colonization in the parenchyma of distant organs, and emergence from dormancy. Some of the genes as outlined above have overlapping functions and therefore can be assigned to several categories.

In the following, we focus on genes promoting lung metastasis. It was demonstrated that clusters of CTCs are oligoclonal precursors of breast cancer metastasis (67). Making use of mammary fat pad implantation of breast cancer cell line MDA-MB-231-2M2 tagged with green fluorescent protein or mCherry, it was shown that CTC clusters consisting of two to 50 cells have a 23- to 50-fold increased metastatic potential to the lungs. In mouse models, knock-down of the cell junction component plakoglobin abrogates CTC cluster formation and lung metastasis. Moreover, presence of CTC clusters in patients with breast- and prostate cancer correlates with a poor prognosis (67).

Transcriptional profiling and subsequent validation experiments making use of metastasizing *versus* non-metastasizing breast cancer cell lines have revealed several genes involved in the process of lung metastasis (68-70). Based on this approach, the lung metastasis gene-expression signature (LMS) was identified, which correlates with worse prognosis and survival in patients with LMS-expressing primary breast tumors (68-70). Part of the LMS are genes such as broad-specificity ligand of the human epidermal growth factor receptor (HER)-family, epiregulin (*EREG*), chemokine C-X-C motif ligand 1 (*CXCL1*), *MMP1* and -2, cell adhesion molecule secreted protein acidic and rich in cysteine (*SPARC*), vascular cell adhesion molecule-1 (*VCAM1*), interleukin 13 decoy receptor *IL13A2*, transcriptional inhibitor of cell differentiation (*ID1*), prostaglandin-endoperoxide synthase (*PTSG2/COX2*) and angiotensin-like 4 (*ANGPTL4*). Some of the identified genes, such as *SPARC*, *VCAM1*, *IL13RA2* and *MMP2* are generally restricted to aggressive lung cancer metastatic cell lines (68). Expression of others, such as *EREG*,

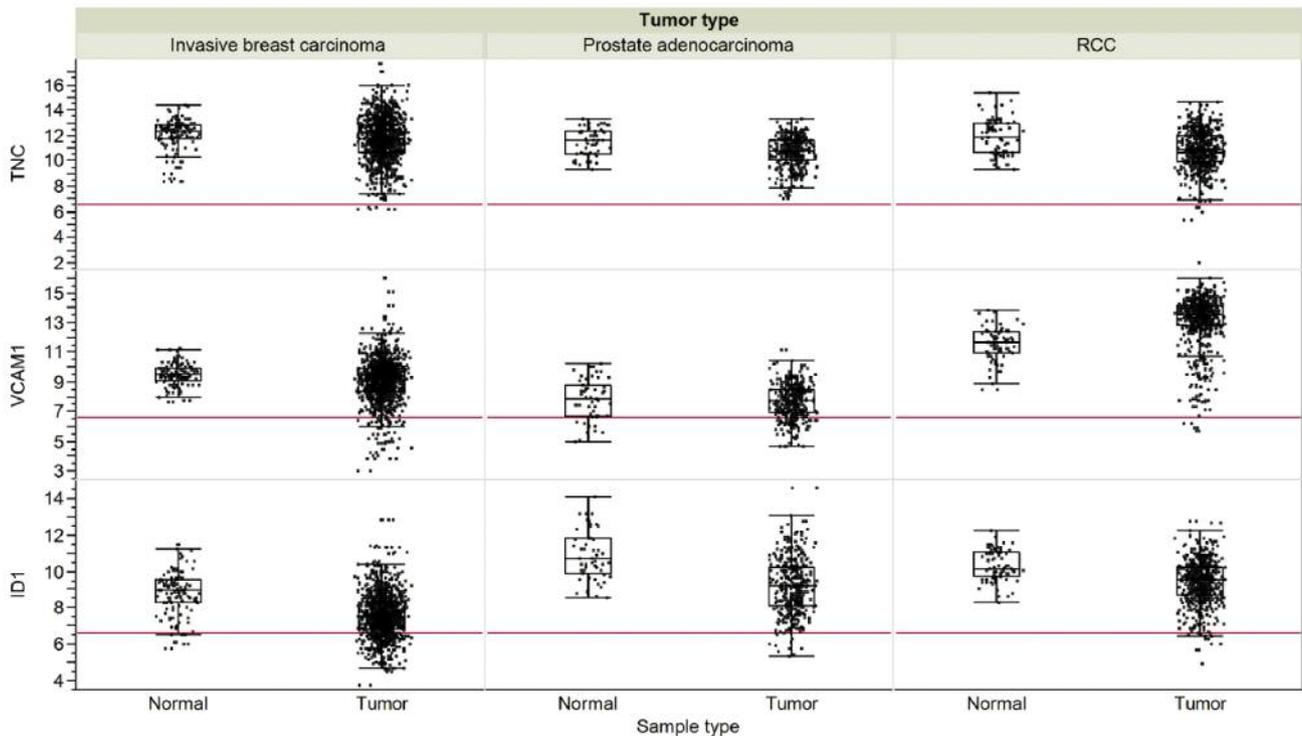


Figure 3. RNA steady-state levels for helix-loop-helix transcription factor modulator *ID1*, vascular cell adhesion molecule 1 (*VCAM1*) and tenascin C (*TNC*) in invasive breast carcinoma, prostate adenocarcinoma and clear cell renal carcinoma (RCC) in comparison to corresponding normal tissues. Invasive breast carcinoma: 111 matched normal, 1,046 tumor samples; prostate adenocarcinoma: 51 matched normal, 360 tumor samples; and RCC: 72 matched normal, 519 tumor samples. Expression was measured by whole-transcriptome sequencing and values provided represent normal read counts (log₂) as derived from the Broad FIREHOSE portal. The red lines indicate low versus higher expression. Expression data are shown as box plots. The line in the middle of the box represents the data median. The rectangles show the upper and lower 25% quartile, therefore 50% of the data points are included in the rectangle, while the whiskers include all other samples except for outliers.

MMP1, *PTSG2*, *CXCL1* and *ID1* are not restricted to lung metastatic cell populations but their levels increase with metastatic propensity. It was shown that lung versus bone tropism of metastasis of breast cancer cell lines rely on different transcriptional programs (69).

Tumors which carry the LMS are larger at diagnosis in comparison to LMS-negative tumors (70). A marked rise in metastasis has been observed for LMS-positive breast tumors which reached 2 cm in diameter, pointing to a mechanistic link between expression of the gene signature, associated tumor growth and metastatic recurrence. Transfection experiments have validated several genes of the LMS as being drivers of lung metastasis of breast cancer cell lines, especially when expressed in combination (68).

Selected genes will be discussed in more detail in the following sections. It is noteworthy that some of the identified genes, such as *EREG*, *PTGS2*, *MMP1* and *ANGPTL4*, cooperate in remodeling the vasculature of mammary tumors and their metastases (68). *ANGPTL4*, a gene induced by TGFβ signaling, was shown to disrupt vascular endothelial cell-cell junctions and mediate retention of disseminated

tumor cells in the lungs (71). As shown in Figure 3, TCGA-based analysis of steady-state levels of RNA for tenascin C, *VCAM1* and *ID1* in breast cancer, RCC and prostate cancer did not reveal up-regulation of the corresponding RNAs by comparison with matching normal tissues, with the exception of *VCAM1* in RCC. The latter finding is probably due to the high vascularization of RCC. To obtain more conclusive data, analysis of patient subgroups and status of lung metastasis have to be performed.

ID Genes

ID genes (*ID1* to *ID4*) and their gene products control differentiation by antagonizing the DNA-binding activities of helix-loop-helix transcription factors (72-74). *ID1* was identified as part of the LMS (68). Rare *ID1*-expressing tumor cells were detected in triple-negative breast cancer (TNBC), but not in other sub-types of breast cancer (75). Regardless of the sub-type; however, *ID1* is expressed in endothelial cells of the breast cancer stroma. *ID1* expression was found to be enriched in TNBC metastases (75). The

functional properties of *ID1/ID3* were investigated in a transgenic model of WNT-driven breast cancer resembling TNBC and in a xenograft model with 2M2-4175 cells, a subpopulation of MDA-MB-231 cells, which metastasize to the lungs. As revealed by knock-down experiments, *ID1/3* are required for initiation of primary tumor formation and for sustained proliferation during early stages of metastatic colonization after extravasation into the lung parenchyma (75). These properties of *ID1/ID3* were mediated both individually as well as in combination. In metastatic foci, coincidence of *ID1* and proliferating cell nuclear antigen expression within tumor cell nuclei was detected (75).

VCAM1

VCAM1 was identified as part of the LMS. The impact of VCAM1 with respect to metastasis is due to facilitation of transendothelial migration of tumor cells into the lungs (76). VCAM1 is expressed on endothelial cells and can initiate transendothelial migration by its clustering and binding to integrins such as $\alpha 4\beta 1$ (very late antigen-4, VLA-4) or $\alpha 4\beta 7$ (77). This results in activation of GTPase ras-related c3 botulinum toxin substrate 1 (RAC1), which induces rearrangement of the cytoskeletal network by remodeling the tight junctions between vascular endothelial cells, thus facilitating transendothelial migration (77, 78) based on recruitment of ezrin to the cytoplasmic tail of VCAM1. In addition, it was observed that VCAM1 expressed on breast cancer cells is able to bind to $\alpha 4\beta 1$ on metastasis-associated macrophages, resulting in phosphoinositide-3 kinase (PI3K)-mediated growth and survival (68).

A clinical correlation between VCAM1 expression in breast cancer cells and relapse in the lungs has been found (79). The prosurvival function of juxtacrine activation of the VCAM1-ezrin-PI3K/akt8 virus oncogene cellular homolog (AKT) pathway can be blocked by antibodies directed against $\alpha 4$ -integrin (68). Natalizumab, a monoclonal antibody directed against $\alpha 4$ -integrin (76, 80), was approved by the US Food and Drug Administration for treatment of relapsing multiple sclerosis and inflammatory bowel disease. Antibodies disrupting $\alpha 4\beta 1$ -VCAM1 interaction might be useful for treatment of metastatic breast cancer.

Colony-stimulating Factor-1 (CSF1) as a Mediator of Lung Metastasis

The role of CSF1 in tumor growth and metastasis was investigated in transgenic mice with mammary tumors induced by PY-MT (81). CSF1 was expressed in the mammary epithelium of PY-MT transgenic mice with *Csf1* null mutation and wild-type transgenic mice. Tumors in CSF1-expressing mice were shown to recruit macrophages and exhibited a highly invasive phenotype with carcinoma

cells invading the stroma and metastasizing to the lungs. In contrast, tumors in *Csf1* knock-down mice were encapsulated, did not break through the basement membrane and did not metastasize to the lungs. Accelerated progression to the late stage of carcinoma was also observed in CSF1-expressing transgenic mice. The underlying mechanism was shown to be based on reciprocal interactions between tumor cells and macrophages (10, 82-84). EGF released by macrophages was shown to induce CSF1 secretion by tumor cells and recruit macrophages by interaction with *CSF1R* (10, 84). Invasion and metastasis is driven by interaction between stromal EGF and EGFR expressed on tumor cells. Increased expression of CSF1 and CSF1R correlates with poor prognosis in patients with breast cancer (85). As shown in Figure 4, steady-state levels for EGF, EGFR, CSF1 and CSF1R were analyzed in invasive breast cancer, RCC and prostate cancer. Significant up-regulation of *CSF1R* was noted in RCC, CSF1 was slightly increased in tumor tissue. In invasive breast carcinoma and prostate adenocarcinoma, no change for these components was observed between tumor tissues and normal tissues. In RCC, *EGF* was down-regulated, but no significant change was observed in invasive breast carcinoma and prostate adenocarcinoma. In prostate adenocarcinoma and RCC, no changes were observed for *EGFR*, which was surprisingly found to be down-regulated in invasive breast carcinoma tissues. For further analysis, patient stratification according to status of lung cancer metastasis and RNA expression of *CSF1*, *CSF1R*, *EGF* and *EGFR* is still required.

CXCL1/2-S100A8/9 Survival Axis

An important driving mechanism for growth, metastasis to the lungs and resistance to chemotherapy was discovered making use of two experimental systems (86). The first was a syngeneic transplant system of cell lines derived from PY-MT-driven mammary tumors and the second one an orthotopic fat pad xenograft model based on LM2-4175 lung metastatic breast cancer cells. It was demonstrated that metastasis and chemoresistance are based on an endothelial carcinoma-myeloid signaling network. Tumor-cell survival-promoting chemokine CXCL1 (87) secreted by tumor cells is able to recruit CD11⁺Gr⁺ myeloid cells into the primary tumor and to disseminated tumor cells in the lung parenchyma based on interaction with CXC chemokine receptor 2 (CXCR2) on myeloid cells (88). Secreted S100 A8/9 derived from the myeloid cells acts as a survival signal for tumor cells in the primary tumor and in the lung parenchyma (89). CXCL1 has emerged among a set of genes whose expression is related to progression and recurrence of breast cancer in the lungs (68). *In situ* hybridization has revealed that the *CXCL1* gene is amplified in 7.5% of primary and 19% of breast cancer metastases (86). The

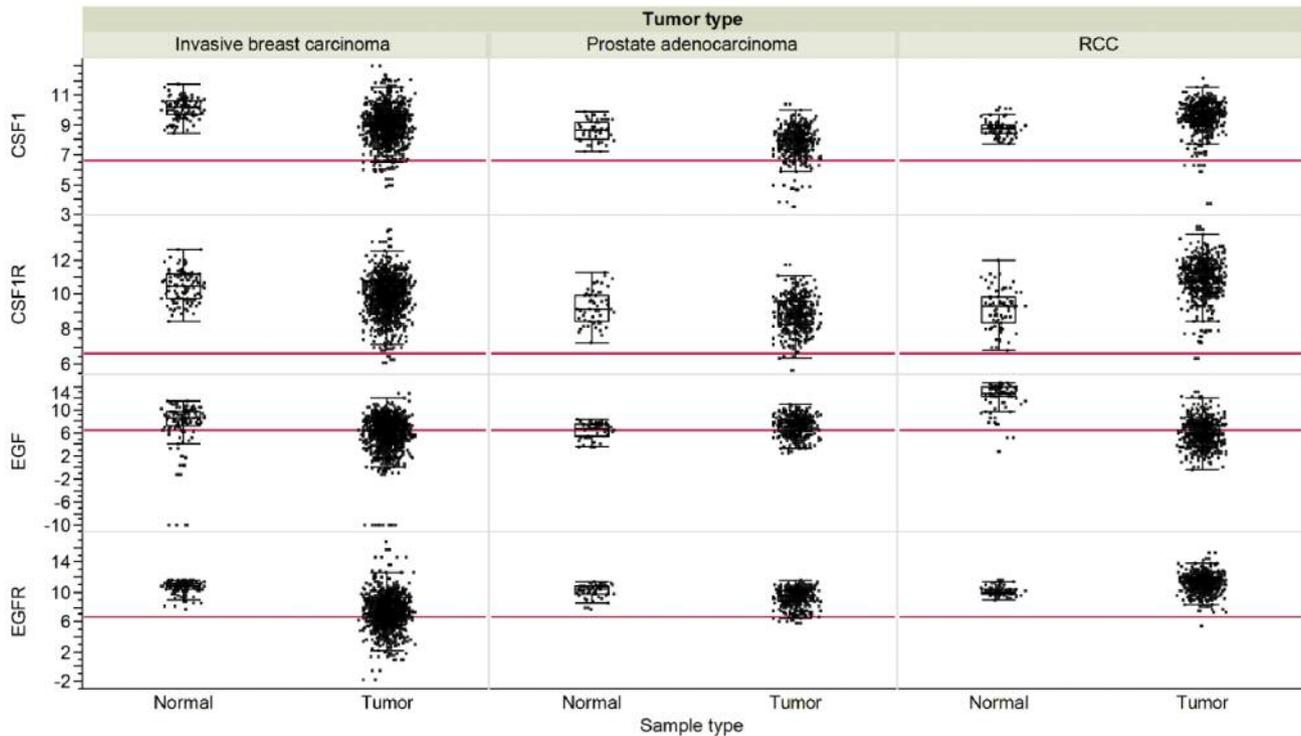


Figure 4. RNA steady-state levels for colony-stimulating factor 1 (CSF1) and its receptor CSF1R, and for epidermal growth factor (EGF) and its receptor EGFR in invasive breast carcinoma, prostate adenocarcinoma and clear cell renal carcinoma (RCC) in comparison to corresponding normal tissues. Invasive breast carcinoma: 111 matched normal, 1,046 tumor samples; prostate adenocarcinoma: 51 matched normal, 360 tumor samples; and RCC: 72 matched normal, 519 tumor samples. Expression was measured by whole-transcriptome sequencing and values provided represent normal read counts (log2) as derived from the Broad FIREHOSE portal. The red lines indicate low versus higher expression. Expression data are shown as box plots. The line in the middle of the box represents the data median. The rectangles show the upper and lower 25% quartile, therefore 50% of the data points are included in the rectangle, while the whiskers include all other samples except for outliers.

survival pathway mediated by CXCL1 was shown to be amplified by TNF α secreted from endothelial cells after treatment with chemotherapeutic agents due to activation of nuclear factor- κ B signaling by TNF α (86). It was shown that CXCR2 blockers can break this cycle, augmenting the efficacy of chemotherapy against tumors and their metastases. CXCR2 antagonists are currently in clinical trials in patients with chronic inflammatory diseases (90) and might also be an option for treatment of breast cancer in combination with chemotherapy.

Concluding Remarks

Most pre-clinical studies addressing the issue of lung tropism of metastasis have focused on breast cancer and its sub-types, fewer studies have investigated melanoma and pediatric sarcoma. It remains to be investigated in more detail which targets are involved in mediating organ tropism of metastasis specifically to the lungs and those which have a general propensity to drive metastasis of different types of tumors to a broader range of distant organs. As outlined, lung tropism of

breast cancer seems to rely on a gene signature (LMS) identified in corresponding primary tumors which does not correlate with metastasis to the bones. Targeting generic drivers of metastasis might be more effective than targeting mediators of dissemination into and colonization of distinct organs because blocking metastasis to defined organs might activate pathways promoting homing to other organs. Prevention of formation of a pre-metastatic niche based on gene signatures predicting metastasis to the lungs might be an effective strategy. However, in order to achieve this objective, reliable markers for the metastatic potential of the corresponding primary tumor should be available and the targets mediating formation of the pre-metastatic niche should be defined and validated in more detail. As outlined previously, targeting of clustered CTCs based on their overexpression of plakoglobin, might be an effective strategy for intervention in metastasis to the lungs and possibly other organs. Since dormant disseminated tumor cells in the lungs are critically dependent on survival pathways, interference with corresponding pathways may improve the efficacy of adjuvant therapy. Inhibition of developmental pathways such as WNT and NOTCH signaling, or reactivation of BMP4 signaling

are possible options in this context. However, routine procedures for detection of dormant metastatic tumor cells in the lungs of patients are not yet established. Since colonization and growth of disseminated tumor cells in the lung parenchyma is triggered by tumor cell–stroma interactions, interference with these types of interactions seems to be a promising avenue. Treatment of established lung metastases is hampered by their resistance to chemotherapy. Identification of specific and homogeneously expressed targets on metastases of the lung and identification of pathways for resistance to chemotherapy might lead to new and more effective approaches.

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