

Metastasis Suppressors and the Tumor Microenvironment

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Abstract The most dangerous attribute of cancer cells is their ability to metastasize. Throughout the process of metastasis, tumor cells interact with other tumor cells, host cells and extracellular molecules. This brief review explores how a new class of molecules – metastasis suppressors – regulate tumor cell–microenvironmental interactions. Data are presented which demonstrate that metastasis suppressors act at multiple steps of the metastatic cascade. A brief discussion for how metastasis suppressor regulation of cellular interactions might be exploited is presented.

Keywords Metastasis suppressor · Nm23 · RECK · BRMS1 · KISS1 · TIMP · E-cadherin · MKK4 · KAI1 · Adhesion · Invasion · MMP · Intravasation · Cohesion · Motility · Endothelium · Angiogenesis · Review

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Introduction

The metastatic cascade describes the process where neoplastic cells in a primary tumor leave their initial location of growth, travel to nearby or distant secondary sites and proliferate into a macroscopic, clinically relevant mass. The process involves intrinsic (i.e., genetic) as well as extrinsic (i.e., tumor cell–microenvironmental signals) factors. In this brief review, our objective is to highlight how metastatic cell interactions with the tumor microenvironment are affected by, or regulated by, metastasis suppressors at various steps of the metastatic cascade. Because of space limitations, we will refer readers to several excellent reviews and primary literature for details on specific topics.

The process of hematogenous and lymphatic metastasis begins when tumor cells attain the ability to break away and grow independently from a primary tumor while migrating through the complex network of proteins, proteoglycans and collagens of the extracellular matrix [1, 2]. These cells then intravasate into the blood or lymphatic vessels where they survive the shear stress of transport [3, 4], resist anoikis [5] and evade immune surveillance [6, 7]. Following vessel transport, metastatic cells may become lodged in capillary beds due to their size or the size of emboli (both homotypic or heterotypic), or adhere to integrins and other, as yet only partly defined, receptors on organ-specific endothelial cells [8–12]. In response to chemoattractants [13], extravasation from vessels at a secondary location follows [14], but is not absolutely essential [15] to proceed to subsequent steps. Following extravasation, metastatic cells must then complete the most crucial, and perhaps most selective, step of the metastatic cascade, proliferation at the secondary site [16–19]. It is important to note that some metastatic cells arrive at a secondary site by means other than those described above: migration across body cavities and peri-vascular transport have been reported for some metastatic cell types. However, regardless of the means of

transport or migration, metastasis is defined by a crucial hallmark of metastatic disease: establishment of a cell mass at a site discontinuous from the primary tumor [18, 19].

Even from this outline, it is clear that tumor cell interactions with the microenvironment are crucial at many steps throughout the metastatic process and contribute to a high level of complexity to what we know about metastasis. Cancer cells are not only dependent on genetic alterations that distinguish them from their normal counterparts, but are also sensitive to extracellular factors that may or may not allow them to metastasize. Tumor cells alter the microenvironment directly (e.g., proteolytic cleavage of the ECM) or indirectly (e.g., induction of nearby cells to secrete proteases) [20]. Conversely, tumor cells can be impacted by stromal cells (e.g., lymphatic or vascular) at each step in the cascade. Thus, it is important for researchers to not only focus on the genetic mutations, deletions or chromosomal translocations within a tumor cell, but also recognize that stromal factors are present and intimately involved in the process. What we learn from these interactions will help to provide more potent therapies targeting metastasis.

We begin with a simple premise – i.e., that some, if not most, of the genetic changes occurring within cancer cells

either manipulate, or are manipulated by, the various microenvironments in which the cells find themselves. The breadth of that hypothesis cannot be covered in the space allowed. So, we will focus this review exclusively upon how changes in the expression of a new class of genes, metastasis suppressors, alter tumor microenvironmental changes.

Metastasis Suppressors

A metastasis suppressor is defined as a molecule whose expression results in the inhibition of a cancer cell's ability to metastasize while having little or no effect on primary tumor growth (reviewed in [21, 22]). This family was first described in the mid-1980's with the discovery of *Nm23*. Since then, more than 20 metastasis suppressors (Table 1) have been reported with more expected to be discovered in years to come. However, although much has been learned, the detailed molecular mechanisms of how these genes act have not been fully elucidated. Their actions are diverse and include the regulation of angiogenesis, extracellular matrix remodeling, transcription regulation and signal transduction. The order of discussion roughly follows the

Table 1 Summary of metastasis suppressors and proposed mechanisms of action

Suppressor	Function(s)	Types of tumor cell–microenvironment interactions affected
BRMS1	Chromatin remodeling; transcription regulation; reduces phosphoinositide signaling; restores gap junctional intercellular communication	Survival in transport, colonization
Cadherins E-Cadherin <i>N</i> -Cadherin Cadherin-11	Cell–cell or cell–matrix adhesion; homotypic cohesion	EMT, invasion
Caspase-8	Induce apoptosis/anoikis	Survival in transport
CD44	Cell–cell or cell–matrix adhesion; bind hyaluronic acid and osteopontin	Migration
DCC	Cytoplasmic architecture; MAPK signaling	Motility, invasion
DLC1	Signaling via Rho-GTPase; cytoskeletal architecture	Migration, invasion
Drg1	Unknown	
Gelsolin	Actin regulatory molecule; cytoskeletal architecture	Migration
KAI1	Bind DARC; integrin interaction; EGFR desensitization	Intravasation, survival in transport
KISS1 (kisspeptins)	Ligand for G-protein coupled receptor	Colonization
MKK4 MKK7 p38	Stress-activated MAPK signaling	Migration, colonization
Nm23	Inhibition of ras signaling; histidine kinase regulation; NDP kinase	Migration, colonization
OGR1	G-protein coupled receptor signaling	Migration, colonization
RhoGDI2	Cytoskeletal architecture; endothelin and neuromedin U signaling	Migration, colonization
RKIP	Raf-MEK signaling; cytoskeletal organization	Migration, invasion
RRM1	PI3K signaling from focal adhesions; cytoskeletal architecture; adhesion	Motility, invasion
SSeCKs	Scaffold for protein kinases; Src signaling, PKC signaling, Rho signaling; VEGF secretion	Angiogenesis, migration
TIMPs	Inhibition of metalloproteinases; signaling	Angiogenesis, migration, invasion, survival in transport, colonization

Not all of the metastasis suppressors listed in this table are discussed in detail. Readers are referred to reviews on metastasis suppressors mentioned in the text for additional details

steps of the metastatic cascade impacted by metastasis suppressors.

E-Cadherin

E (epithelial)-cadherin is one of the most studied proteins in cancer progression and metastasis. A transmembrane glycoprotein, E-cadherin binds homotypically to E-cadherin on neighboring cells through Ca^{+2} -dependent extracellular domains. E-cadherin anchors to actin filaments in the cytoplasm through structural interactions with α - and β -catenin. This acts to promote cellular polarity in epithelial layers by cytoskeletal organization. Regulation of E-cadherin occurs via multiple mechanisms, including receptor tyrosine kinases, transforming growth factor beta (TGF- β) and NF κ B signaling (reviewed in [23]) as well as DNA methylation [24, 25].

Downregulation of E-cadherin expression is known to induce (or is a consequence of) an epithelial-to-mesenchymal transition (EMT), a process in which epithelial cells revert to a more motile mesenchymal phenotype believed to play a major role in the invasion and metastasis of cancer cells [26–29]. The loss of surface E-cadherin protein, resulting in a loss of polarity and reduced association with other epithelial cells, is a hallmark of EMT. EMT itself is a known cellular process in the maturing embryo where cells migrate to developing regions of an organism, but induction of EMT in adult cells is believed to play a negative role when cancer cells employ this process to enhance their invasiveness [26–29].

Shortly after the initial discovery and description of the cadherin family, a number of studies reported expression of E-cadherin to be inversely correlated to invasive qualities in cancer cells and that transfection of E-cadherin into invasive cell lines reduced their malignancy [30]. A continually growing cohort of clinical and experimental data confirms the anti-metastatic capabilities of E-cadherin [31] and significance of EMT [32, 33].

As cells growing in an epithelial layer begin to lose contact with themselves (cohesion) they often begin to produce cellular protrusions through reorganization of the cytoskeleton and plasma membrane that form new contacts with the surrounding microenvironment [1, 2, 34]. These new adhesive contacts promote the migration of cells through the extracellular matrix and plays on a theme that as cancer cells progress to a more metastatic phenotype, they become more independent and less reliant on other cells for survival signals and growth factors (loss of gap junction communication, anoikis, etc.). By continuing to further delineate the multiple molecular pathways involved in the induction of EMT and loss of E-

cadherin, a better understanding of how cells may be inhibited in these processes will lead to more adept anti-metastatic therapies.

However, it is still not certain that EMT is required for metastasis [35–37]. For example, recent data have identified different mechanisms by which tumor cells can invade surrounding tissues and extracellular matrices (reviewed in [1]). Many carcinomas migrate as clusters of cells, suggesting that they have maintained vestigial epithelial–epithelial interactions (perhaps, though not directly tested to our knowledge, maintaining E-cadherin expression). The EMT-based motility refers to migration of individual cells in a manner dependent upon proteolysis; whereas, individual cell movement independent upon proteases – the so-called epithelial–amoeboid transition – is becoming increasingly recognized.

SSeCKS

Src Suppressed C Kinase Substrate (SSeCKS) was discovered using PCR based subtractive hybridization in NIH3T3 mouse fibroblasts and found to be suppressed by oncogenic forms of *src*, *ras*, *fos*, and *myc* [38]. *SSeCKS* mRNA was found to accumulate in non-dividing cells, while *SSeCKS* mRNA was suppressed in dividing or transformed cells. *SSeCKS* protein is phosphorylated by protein kinase C (PKC) and involved in cytoskeletal architecture by binding F-actin and mediating signaling pathways [38, 39]. *SSeCKS* was first described as a metastasis suppressor when its expression was able to inhibit the ability of MatLyLu rat prostate cancer cells to metastasize to lungs in nude mice after flank injection but had minimal effect on overall tumorigenicity of the cells. This experimental evidence was supported by clinical data showing *SSeCKS* expression in benign and well differentiated prostate carcinomas, but exhibited loss of expression in highly aggressive and undifferentiated prostate lesions [40].

The molecular mechanisms reported for SSeCKS, thus far, involve a complex signaling activation sequence that can be initiated by extracellular factors. The binding of growth factors and adhesion of integrins to proteins of the extracellular matrix have both been shown to activate multiple signaling and phosphorylation cascades, leading to cytoskeletal reorganization and cell migration mediated through signaling molecules such as Src and PI3K. SSeCKS, in its dephosphorylated state, acts as a scaffolding protein where it binds signaling molecules such as PKC, PKA, calmodulin and cyclins. Upon mitogenic signaling, SSeCKS is phosphorylated and translocates to the perinuclear membrane releasing its bound signaling molecules to mediate changes within a cell [41].

Most interesting may be SSeCKS involvement in focal adhesions and their combinatorial roles on cytoskeletal rearrangement. Focal adhesions are formed at the plasma membrane when receptors or integrins bind extracellular matrix proteins and induce clustering of actin and other protein complexes at these sites. Focal adhesion kinase (FAK) is a protein tyrosine kinase recruited to focal adhesions. Phosphorylation of SSeCKS through stimulation by multiple growth factors has been shown to be FAK dependent through mediation of an unidentified FAK induced kinase [42]. This FAK-dependent phosphorylation of SSeCKS not only induces its release of signaling molecules, but reduces its F-actin binding capability. Thus, SSeCKS acts to sequester growth factors and binds F-actin when in its dephosphorylated state during G0 and early G1 phases. But upon mitogenic signaling SSeCKS relinquishes actin binding and allows induction of signaling cascades leading to changes in the cytoskeleton.

The effects of SSeCKS as a metastasis suppressor can be best appreciated if SSeCKS acts as a regulator of cytoskeleton reorganization and migration. Lack of expression or loss of function, could promote the migration of primary tumor cells to a secondary site by allowing a more motile phenotype.

Nm23

Nm23 (non-metastatic clone 23) gene was discovered by screening K1735 murine melanoma cells with varying metastatic capability in an attempt to identify metastasis related genes differentially expressed between high and low metastatic cells [43]. Two of the poorly metastatic cell lines had a 10-fold higher expression of *Nm23* mRNA than the other five related highly metastatic lines. Transfection of *Nm23* into the highly metastatic cell clones still allowed tumor growth but metastasis was inhibited, thereby fulfilling the definition of a metastasis suppressor. Prior to the discovery of *Nm23*, it was shown that when rat embryo fibroblasts (REFs) were transformed with the human oncogene *c-Ha-ras* or co-transfected with *c-Ha-ras* and the adenoviral type 2 E1a gene, both groups formed tumors when injected subcutaneously (s.c.) into athymic mice. However only *c-Ha-ras* transfected cells formed metastatic lung nodules, while the E1a co-transfected cells formed significantly fewer lung metastases [44]. Subsequently, it was found that *Nm23* was upregulated in the REF cells by the E1a gene, consistent with a role of *Nm23* as a metastasis suppressor. The anti-metastatic activities of *Nm23* have since been demonstrated in multiple cancer models (reviewed in [45, 46]) and *Nm23* expression inhibits invasion, motility, and anchorage independent growth in vitro [47]. *Nm23-H1* (human isoform 1; there are now eight known human *Nm23*

isoforms) has been correlated to clinical data in multiple tumor types [45, 46]. Low levels of *Nm23* mRNA expression have been associated with good patient prognosis and reduced metastases. Although the preponderance of evidence supports *Nm23-H1* as a metastasis suppressor, conflicting clinical reports suggest the possibility that the actions of *Nm23-H1* are cell and tissue type specific [48]. These findings may be due to multiple isoforms, homologs and other regulatory mechanisms of *Nm23* (reviewed in [49, 50]). Efforts to characterize the tumorigenic and metastatic implications of other *Nm23* homologs are currently underway [47].

Data suggesting possible *Nm23* interactions with the microenvironment have been reported, but a precise mechanism for how *Nm23* affects cell migration and motility still remain unclear. Numerous reports link *Nm23-H1* with the Ras family of proteins. *Nm23-H1* was shown to bind and inhibit the activity of Tiam1, a guanine exchange factor (GEF) downstream of Ras that activates Rac1, a small GTP binding protein that leads to proliferation and migration by cytoskeletal reorganization [51]. Data also exist correlating involvement in the upregulation of matrix MMP-9 in B-cell lines when *Nm23-H1* function was altered by the Epstein Barr virus latent protein EBNA3C [52]. Conversely, transfection of *Nm23* in the L9981 human non-small cell lung cancer cell line decreased levels of MMP2 [53].

More recent data shows a novel link in the *Nm23-H1* mediated suppression of metastasis. Using gene expression microarrays, comparison of cancer cells transfected to over-express wild-type *Nm23-H1* with MDA-MB-435 expressing mutant forms of *Nm23-H1*, nine genes were down-regulated by wild-type *Nm23* and correlated to human breast cancer cohorts [54]. Specifically, EDG2 (endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor 2), a lysophosphatidic acid (LPA) receptor restored motility when overexpressed in *Nm23-H1* suppressed cells. Knock down of EDG2 by siRNA in metastatic cells reduced their motility. These findings fit well with previous data involving *Nm23-H1* and G-proteins in that LPA is a potent signaling molecule which is mitogenic and activates the GTPase Rho. The latter initiates cytoskeletal reorganization and induces cellular migration. Further examination of these findings and characterization of the regulation of EDG2 by *Nm23* may provide new insight into a more detailed description of how *Nm23* controls cellular responses from the microenvironment.

TIMPs

The tissue inhibitors of metalloproteinases are a family of four endogenously expressed, secreted protease inhibitors, TIMP1–4, that inhibit the function of MMPs (matrix

metalloproteinases). Due to their nature of action, TIMPs can inhibit the remodeling and plasticity of the extracellular environment caused by MMPs. In the context of metastasis, early reports showed human prostate tissue samples exhibited higher expression levels of MMP2 and MMP9 and reduced TIMP levels in malignant tissues compared to normal prostate and breast tissue. Experimental studies confirmed clinical data showing that levels of TIMP1 correlated inversely to metastatic potential in colon carcinoma cell lines with differing metastatic potential [55]. Transfection of TIMP2 into human lung carcinoma cell lines decreased colony formation in soft agar and invasion in Matrigel [56]. The anti-invasive properties from expression or treatment with TIMPs has continued to be observed in models of cancer and correlates to their role as metastasis suppressors.

However, the balance of MMPs and TIMPs is not as straightforward as originally anticipated. While many studies have shown a decrease in TIMPs in metastatic disease some experimental models suggest TIMPs increase cancer cell invasion. At the forefront of these discrepancies is the complexity of the MMP2, TIMP2, MT1-MMP (MMP14) axis. TIMP2 binds MMP2 in its latent form. This complex can then associate with MT1-MMP and facilitate the activation of MMP2, thus providing a role for TIMP2 in the promotion and activity of MMP2 (reviewed in [57]).

It is clear that TIMPs can act to suppress the invasive effects of MMPs in both clinical samples and experimental models; however, there is now believed to be a higher level complex system involving cooperation of various MMPs and TIMPs. So, their actions must be further studied to determine the exact pathways involved. In addition, it is likely that there is a high involvement of stromal cells influencing the actions of MMPs and TIMPs and these interactions must be considered when examining levels of MMPs/TIMPs in cancer cells and clinical samples.

RECK

RECK (*reversion-inducing cysteine rich protein with Kazal motifs*) was originally identified using a cDNA library screen on v-Ki-RAS transformed fibroblasts where it exerted a flat morphology phenotype (i.e., revertant) [58]. RECK mapped to chromosome 9, and its protein product is a membrane bound glycoprotein capable of inhibiting the secretion of MMP9, invasion and metastasis [58]. RECK is downregulated in many oncogenic transformed cell lines, while forced expression of RECK in metastatic cell lines leads to a reduction in invasion and metastasis [59]. RECK is expressed in most normal cells and proven as a prognostic marker for patient outcome in hepatocellular,

prostate, breast, colorectal and non-small cell lung carcinomas [59–61]. RECK also inhibits the activity of MMP-2 and membrane-type (MT)-MMP1 [59, 62]. This suggests a role for RECK in regulating interactions of MMPs, which have numerous roles in most steps of the metastatic cascade [20, 63].

The transcriptional regulation of RECK has been investigated and involves the Sp1 family of transcription factors, histone deacetylation and methylation and are discussed elsewhere [59, 64–66]. What are the direct implications of RECK dependent downregulation on activity of MMPs, and how does this relate to tumor progression and metastasis? Building evidence links MMP-9 and MMP-2 to angiogenesis and invasion. When HT1080 fibrosarcoma cells were transfected to express RECK and injected into nude mice, no changes in tumor size were noted. However as tumors grew, RECK expressing tumor vessel density and vessel sprouting were reduced. Both changes lead to longer survival and less vascularized tumors [67]. This was confirmed in clinical samples of human esophageal squamous cell carcinoma. RECK expression decreased during tumor progression and was inversely correlated to lymph node metastases, vascular endothelial growth factor (VEGF) and endoglin (CD105) expression [68]. RECK, therefore, appears to be a regulator of angiogenesis, perhaps through MMP regulation.

KAI1

KAI1/CD82 was discovered after its ability to suppress metastasis when transfected into the prostate cancer cell line AT6.1 and was mapped to chromosome 11p11.2 [69]. The expression of *KAI1* is downregulated in numerous cancer cell lines and its metastasis suppressive capability has been strongly correlated with clinical data from a variety of human cancers including non-small cell lung carcinoma, pancreatic, bladder and breast cancers [70, 71].

The *KAI1* protein is a member of the tetraspanin superfamily subject to post translational modifications including glycosylation and palmitoylation. Since its discovery *KAI1* has been shown to interact with many proteins, including other tetraspanins, integrins, growth factor receptors, uPAR, KITENIN and protein kinase C (PKC), while its transcriptional regulation is affected by NF κ B, p53, β -catenin and other transcriptional factors downstream of MAPK pathways (reviewed in [70]). Collectively, expression of *KAI1* leads to a decrease in cellular motility and has been shown to induce apoptosis and reduce anchorage independent growth in some models. Additionally, *KAI1* has been demonstrated to decrease protein and mRNA levels of MMP9, while increasing TIMP1 levels when transfected into the human lung carcinoma cell line H1299 [72]. All of

these activities are likely to be involved in the regulation of cell migration, but a unified theory of how KAI1 inhibits metastasis, or where in the metastatic cascade KAI1 acts, had yet to be determined.

An important discovery in the function of KAI1 was recently made. Using a normal human prostate cDNA library, a yeast two-hybrid screen with *KAI1* cDNA identified KAI1 interactors, including strong binding affinity with Duffy antigen receptor for chemokines (DARC) [73]. DARC is highly expressed in vascular and lymphatic endothelium of the prostate while little DARC was found on the epithelium and stroma. Cell–cell binding assays showed KAI1 on AT6.1 prostate cancer cells were able to bind when overlain onto endothelial cells expressing DARC. KAI1-expressing cells binding DARC had reduced DNA synthesis and were made senescent in prostate, breast and lung cancer cell lines. Similar growth inhibition and senescence were not observed for KAI1-expressing cells in *Darc*^{-/-} mice, concomitant with failure to reduce metastasis. Based upon these findings, KAI1 expressing cells are rendered incapable of proceeding or are eliminated when leaving the primary tumor (i.e., intravasation into the blood or lymphatic vessels).

BRMS1

The observation that loss of genetic material on chromosome 11 occurs in many metastatic breast carcinomas lead to the discovery of Breast Cancer Metastasis Suppressor 1 (BRMS1). Microcell-mediated transfer of chromosome 11 into MDA-MB-435 suppressed metastasis to the lungs and regional lymph nodes by >95% while minimally affecting orthotopic growth in the mammary fat pad. Differential display identified BRMS1, which mapped to 11q13.1–11q13.2. Structural analysis of the encoded protein identified multiple coiled coil domains, an imperfect leucine zipper motif, and putative phosphorylation and nuclear localization signal sites, suggesting protein–protein interactions and possible transcriptional regulation [74]. BRMS1 metastasis suppressing ability has extended to multiple xenograft and syngeneic models [75, 76].

The clinical correlates of BRMS1 are more complex. Since antibodies recognizing BRMS1 have only recently been developed, most studies have relied on mRNA expression. The correlations have been mixed, showing loss of BRMS1 mRNA correlating with increased tumor grade and poor prognosis [77] or high levels of BRMS1 mRNA correlated with better prognosis and patient outcome [78–80]. In other studies, a lack of correlation exists between BRMS1 mRNA levels and axillary lymph node metastases in breast cancer samples [81]. In the first, and as-yet only, published study examining BRMS1 protein,

reduced BRMS1 expression correlated to worsened disease free survival in a subset of cancer patients with over-expression of *Her2*, invoking possible interactions of BRMS1 with oncogenic signaling [82]. Importantly, the data from clinical specimens highlights a known, but often under-appreciated, complication that mRNA and protein levels do not necessarily correlate. In the case of BRMS1, we have shown profound regulation of BRMS1 protein at a post-translational level [83]. Therefore, we emphasize caution when interpreting mRNA data.

Since its discovery, mechanistic insights into BRMS1 function have contributed to (but unfortunately somewhat confused) our understanding of how BRMS1 may suppress metastasis. BRMS1 interacts with several large multi-subunit mSin3-histone deacetylase (HDAC) enzyme complexes that remove acetyl groups from histone tails changing chromatin structure and correspondingly transcription [84]. Thus, BRMS1 is positioned to regulate a cadre of genes controlling multiple steps in the metastatic cascade. This speculation has been borne out in gene expression profiling [85].

With regard to BRMS1 effects on tumor cell interactions with the tumor microenvironment and extracellular matrix, BRMS1 interacts directly with the nuclear factor-kappa B (NFκB) transcription factor, NFκB consists of a transcription factor heterodimer composed of p50 and p65 subunits maintained in an inactive state in the cytoplasm by physical interaction with the inhibitory regulatory protein IκB. Phosphorylation of IκB by the IκB-alpha kinase (IKK) complex frees NFκB by exposing nuclear localization signals allowing it to translocate and regulate transcription of genes. NFκB is upregulated or constitutively activated in numerous human cancers. One of NFκB's downstream targets is the serine protease, urokinase-type plasminogen activator (uPA). Active uPA cleaves plasminogen in its inactive zymogen state to its active form, plasmin. Active plasmin initiates multiple enzymatic cascades, depending on the microenvironment of a cell. In MDA-MB-231 human breast and C8161.9 human melanoma cell lines, which both exhibit constitutively active NFκB, expression of BRMS1 inhibited the TNFα-induced phosphorylation of IκB and expression of BRMS1 with a concomitant decrease in uPA. Additionally, BRMS1 acting as a co-repressor with HDAC1 has been shown to directly interact with the RelA/p65 subunit of NFκB and cause deacetylation of lysine 310. The removal of the acetyl groups decreases the transactivation potential of RelA/p65 and, thus, decreases the action of NFκB in non-small cell lung carcinoma (NSCLC) cells [86]. Interestingly, when siRNA was used to knockdown BRMS1, the lack of deacetylation by HDAC1 on RelA/p65 led to NFκB chromatin binding to the promoters of two anti-apoptotic genes, *cIAP2*, *Bfl-1/A1* suggesting that loss of BRMS1 may lead to a more apoptosis resistant phenotype [86].

Recent data confirm that BRMS1-expressing cells are more sensitive to anoikis than their metastatic counterparts [87]. As a result, one would predict reduced survival of BRMS1 cells in the circulation. Phadke et al. [87] and Hedley and colleagues (BRMS1 suppresses breast cancer metastasis in multiple organs by reducing solitary cell survival and inhibiting initiation of growth. B.D. Hedley, K.S. Vaidya, P. A. Phadke, L. MacKenzie, D.W. Dales, C.O. Postenka, I.C. MacDonald, A.F. Chambers, manuscript in preparation) have used green fluorescent protein-tagged tumor cells to quantify the fates of injected tumor cells. BRMS1-expressing human breast carcinoma cells were indeed less likely to reach lungs, bone and liver following direct injection into afferent vessels, consistent to increased sensitivity to anoikis.

Another possible role for BRMS1-mediated interactions with the microenvironment involve data in which BRMS1 expression restored gap junctional intercellular communication via regulation of connexin expression. Connexins are monomeric proteins (typically named by their molecular mass) that form hexamers, known as connexons, that insert into the plasma membrane. Connexons physically connect to connexons on other cells to provide direct communication between cells via transfer of molecules <1.5 kDa, e.g., cAMP, PIP3 and various ions [88]. Connexons are sensitive to levels of Ca^{2+} , pH, cAMP levels. Loss of gap junction communication between cells occurs during neoplastic progression. Using calcein and lucifer yellow fluorescent dye transfer assays, homotypic gap junction communication was restored in BRMS1-expressing MDA-MB-435 cells. This change was apparently mediated by BRMS1 regulation of individual connexin proteins [89, 90]. Additionally, in studies examining GJIC between MDA-MB-435 cells and the human osteoblastic cell line hFOB1.19, MDA-MB-435 cells communicated via gap junctions at a greater level with the osteoblasts. BRMS1-expressing cells maintained homotypic communication but lost heterotypic communication with osteoblasts, which may have implications explaining how metastatic breast cancer cells communicate and survive at secondary sites such as bone [91].

The heterotypic GJIC may explain other studies by Phadke et al. [87], who showed that, in addition to increasing sensitivity to anoikis, BRMS1 cells successfully seeding at ectopic sites remained dormant for extended times. At minimum BRMS1 appears to control how tumor cells respond to growth signals differentially (i.e., grow in mammary fat pads but not lungs or bone). Such a difference in growth response can explain why tumor cells expressing metastasis suppressors form primary tumors, but not secondary tumors/metastases.

Another molecule regulated by BRMS1 is the secreted extracellular matrix protein osteopontin (OPN). OPN expression is directly correlated to aggressive behavior in multiple tumor types. When BRMS1 is re-expressed, OPN levels

drop by >75%. Recent data from a collaborative project to Ben Hedley and Ann Chambers lab, restoration of OPN expression in BRMS1-expressing MDA-MB-435 cells restores metastatic competency (Re-expression of Osteopontin in Breast Cancer Metastasis Suppressor 1-Expressing Breast Cancer Cells Restores Metastatic Potential. B.D. Hedley, D.R. Welch, A.L. Allan, W. Al-Katib, D.W. Dales, C.O. Postenka, I.C. MacDonald, A.F. Chambers, manuscript submitted). Clearly, loss of OPN is a downstream effect of BRMS1. Given its secretion into the tumor cell milieu, it is reasonable to assume that it may change the microenvironment. However, direct testing of this hypothesis has not been done.

MKK4

Evidence for another metastasis suppressor on chromosome 17 was suggested by microcell-mediated chromosome transfer into the highly metastatic AT6.1 rat prostate cancer cell line [92] and subsequent narrowing of the region at a locus away from Nm23 [93]. Iterative mapping studies identified Mitogen Activated Protein Kinase Kinase 4 (MKK4) as the metastasis suppressor [93–95]. Histologic analyses of human prostate [96] and ovarian [97] cancer samples confirmed an inverse relationship between MKK4 and cancer progression.

Mechanistic insights were first derived from ovarian xenograft models. SKOV3ip.1 ovarian carcinoma cells (which lack endogenous MKK4 expression but maintain all other functional components both up and downstream of MKK4) were inhibited for metastasis by >90% when MKK4 was ectopically expressed [97]. Because the molecular and biochemical functions of MKK4 place it in multiple signal transduction pathways by acting as a dual specificity kinase involved in the activation of c-Jun NH2-terminal protein kinase (JNK) and p38, the precise actions of how MKK4 inhibits metastasis were still not clearly defined. Both JNK and p38 are activated by stress signals and regulate diverse processes such as apoptosis and cellular proliferation. Subsequent evidence has begun to delineate the multiple actions of MKK4 through JNK and p38 with modulation by MKK7 and MKK6, respectively [98–100].

Examination of MKK4-expressing ovarian carcinoma cells revealed that they seed peritoneal surfaces with high frequency, but do not form macroscopic colonies efficiently [98]. Those observations are consistent with observations that the ratio between p38 signaling and ERK signaling determine proliferative versus dormant states among cancer cells [101, 102]. The implication is that these pathways are differentially impacted by exogenous signals, however the precise molecules involved have not yet been defined.

KISS1

The KISS1 metastasis suppressor was found by subtractive hybridization comparing matched metastasis competent C8161 human melanoma cells to metastasis suppressed chromosome 6-C8161 cell hybrids [103]. KISS1, located on chromosome 1q32, inhibits metastasis in melanoma, breast, and ovarian cancer xenograft models (reviewed in [104]). In clinical samples, KISS1 mRNA levels are inversely correlated to invasiveness in multiple human cancers (reviewed in [104]).

In the C8161 human melanoma xenograft model, cells seeded in the lung, but were unable to proliferate into a macroscopic tumor [105]. The mechanism by which KISS1 prevents colonization remains unclear. However, a major breakthrough toward understanding KISS1 function was made by the work of three independent laboratories showing that KISS1 contains a secretion signal, is proteolytically processed to make so-called kisspeptins, and secreted [106–108]. Specifically, binding of a 54 amino acid kisspeptin, called metastin, to a G-protein orphan receptor, GPR54, suggested an autocrine feedback loop. Nash and colleagues confirmed that secretion was necessary for the KISS1 anti-metastatic activity. However, they observed something surprising. None of the tumor cells suppressed for metastasis expressed GPR-54, questioning whether the ligand-receptor activation is necessary at all. Although evidence of another receptor was not found (unpublished), the authors have speculated that a tissue-specific paracrine feedback loop may be operational [104].

Summary and Perspectives

If no other observation were taken into account, the fact that metastasis suppressors allow growth in orthotopic sites but prohibit growth at ectopic sites highlights their central role in how tumor cells interact with the myriad microenvironments encountered during the metastatic cascade. Indeed, the evidence presented in this minireview describe functions for a subset of metastasis suppressors and how each suppressor impacts interactions between tumor cells, matrices and other cell types. What emerges is a picture in which tumor cell expression of specific genes regulate success or failure at each step of the metastatic cascade.

When tumor cells express molecules on their cell surfaces (e.g., E-cadherin) which promote cell–cell adhesion or molecules that prohibit their motility and invasion (e.g., Nm23, TIMPs, SseCKS), they are more apt to remain at the primary tumor. Likewise, molecules that affect recruitment and structural aspects of angiogenic vessels could impact tumor cell dissemination. Still other suppressors reduce cell survival during transit (e.g., caspase-8,

BRMS1, KAI1). We have not even mentioned the multitude of immunogenic molecules that make circulating tumor cells visible to the immune system.

Perhaps most importantly, a growing number of metastasis suppressors, highlighted by KISS1, MKK4, p38 and MKK7 in this review, control the last step of the metastatic process, colonization. When tumor cells arrive at various secondary sites, they find themselves in a milieu of growth promoting and growth inhibitory factors that are different from their site of origin. Depending upon how the cells respond to competing signals, they will either die, remain quiescent or proliferate. It is only when they proliferate that they will become a *bona fide* metastases. We speculate that a subset of metastasis suppressors will eventually determine in which organs metastases develop. Those molecules would be responsible for the predilection for metastasis observed in many tumor types (e.g., bone metastases from breast and prostate carcinomas).

We find ourselves at the earliest stages of a research endeavor that, at its root, defines cellular responses in complex mixtures of molecules. It has been clear, and the results presented in this short review emphasize, that tumor cells alone do not determine whether metastases develop. It is clear that the response of tumor cells to signals from the many microenvironments through which it traverses to seed and proliferate a secondary site are essential for metastasis. Experiments to elucidate the mechanisms of metastasis are currently relatively basic since they explore only one factor at a time. Significant advances will require a sophisticated and more comprehensive mimicry of the various microenvironments, including growth factors, growth factor ratios, growth inhibitors, matrices and biophysical parameters such as shear and torsion.

Our objective in writing this review has been to highlight the interactions between tumor cells and various stages of the microenvironment by describing how a selected number of metastasis suppressors control key interactions. We hope that the manner in which we have presented the facts stimulates investigators studying metastasis to incorporate elements of integral cell–cell and cell–matrix interactions.

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References

1. Wolf K, Wu YI, Liu Y et al (2007) Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. *Nat Cell Biol* 9:893–904
2. Friedl P, Wolf K (2003) Tumour-cell invasion and migration: Diversity and escape mechanisms. *Nat Rev Cancer* 3:362–374

3. Weiss L, Orr FW, Honn KW (1989) Interactions between cancer cells and the microvasculature: A rate regulator for metastasis. *Clin Exp Metastasis* 7:127–167
4. Weiss L (1990) Metastatic inefficiency. *Adv Cancer Res* 54:159–211
5. Rennebeck G, Martelli M, Kyprianou N (2005) Anoikis and survival connections in the tumor microenvironment: Is there a role in prostate cancer metastasis? *Cancer Res* 65:11230–11235
6. Wang SL, El-Deiry WS (2003) TRAIL and apoptosis induction by TNF-family death receptors. *Oncogene* 22:8628–8633
7. Fidler IJ (1974) Immune stimulation-inhibition of experimental cancer metastasis. *Cancer Res* 34:491–498
8. Hehlgans S, Haase M, Cordes N (2007) Signalling via integrins: Implications for cell survival and anticancer strategies. *Biochim Biophys Acta Rev Cancer* 1775:163–180
9. Cavallaro U, Christofori G (2001) Cell adhesion in tumor invasion and metastasis: loss of the glue is not enough. *Biochim Biophys Acta Rev Cancer* 1552:39–45
10. Pauli BU, Augustin-Voss HG, El-Sabban ME et al (1990) Organ-preference of metastasis. The role of endothelial cell adhesion molecules. *Cancer Metastasis Rev* 9:175–189
11. Nicolson GL (1988) Organ specificity of tumor metastasis: role of preferential adhesion, invasion and growth of malignant cells at specific secondary sites. *Cancer Metastasis Rev* 7:143–188
12. Hart IR (1982) “Seed and soil” revisited: mechanisms of site-specific metastasis. *Cancer Metastasis Rev* 1:5–16
13. Ruffini PA, Morandi P, Cabioglu N et al (2007) Manipulating the chemokine–chemokine receptor network to treat cancer. *Cancer* 109:2392–2404
14. Naumov GN, MacDonald IC, Chambers AF et al (2001) Solitary cancer cells as a possible source of tumour dormancy? *Semin Cancer Biol* 11:271–276
15. Al Mehdi AB, Tozawa K, Fisher AB et al (2000) Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: a new model for metastasis. *Nat Med* 6:100–102
16. Townson JL, Chambers AF (2006) Dormancy of solitary metastatic cells. *Cell Cycle* 5:1744–1750
17. Chambers AF, Groom AC, MacDonald IC (2002) Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer* 2:563–572
18. Eccles SA, Welch DR (2007) Metastasis: recent discoveries and novel treatment strategies. *Lancet* 369:1742–1757
19. Welch DR (2007) Do we need to redefine a cancer metastasis and staging definitions? *Breast Disease* 26:3–12
20. Chambers AF, Matrisian LM (1997) Changing views of the role of matrix metalloproteinases in metastasis. *J Natl Cancer Inst* 89:1260–1270
21. Vaidya KS, Welch DR (2007) Metastasis suppressors and their roles in breast carcinoma. *J Mamm Gland Biol Neopl* 12:175–190
22. Rinker-Schaeffer CW, O’Keefe JP, Welch DR et al (2006) Metastasis suppressor proteins: Discovery, Molecular mechanisms and Clinical Application. *Clin Cancer Res* 12:3382–3389
23. Guarino M, Rubino B, Ballabio G (2007) The role of epithelial–mesenchymal transition in cancer pathology. *Pathology* 39:305–318
24. Graff JR, Gabrielson E, Fujii H et al (2000) Methylation patterns of the E-cadherin 5′ CpG island are unstable and reflect the dynamic, heterogeneous loss of E-cadherin expression during metastatic progression. *J Biol Chem* 275:2727–2732
25. Graff JR, Greenberg VE, Herman JG et al (1998) Distinct patterns of E-cadherin CpG island methylation in papillary, follicular, Hurthle’s cell, and poorly differentiated human thyroid carcinoma. *Cancer Res* 58:2063–2066
26. Hugo H, Ackland ML, Blick T et al (2007) Epithelial–mesenchymal and mesenchymal–epithelial transitions in carcinoma progression. *J Cell Physiol* 213:374–383
27. Potter JD (2007) Morphogens, morphostats, microarchitecture and malignancy. *Nat Rev Cancer* 7:464–474
28. Hay ED (2005) The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create it. *Dev Dyn* 233:706–720
29. Thiery JP (2002) Epithelial–mesenchymal transitions in tumour progression. *Nat Rev Cancer* 2:442–454
30. Foty RA, Steinberg MS (2004) Cadherin-mediated cell–cell adhesion and tissue segregation in relation to malignancy. *Int J Dev Biol* 48:397–409
31. Kashima T, Nakamura K, Kawaguchi J et al (2003) Over-expression of cadherins suppresses pulmonary metastasis of osteosarcoma in vivo. *Int J Cancer* 104:147–154
32. Puisieux A, Valsesia-Wittmann S, Ansieau S (2006) A twist for survival and cancer progression. *Br J Cancer* 94:13–17
33. Yang J, Mani SA, Weinberg RA (2006) Exploring a new twist on tumor metastasis. *Cancer Res* 66:4549–4552
34. Weaver AM (2006) Invadopodia: specialized cell structures for cancer invasion. *Clin Exp Metastasis* 23:97–105
35. Christiansen JJ, Rajasekaran AK (2006) Reassessing epithelial to mesenchymal transition as a prerequisite for carcinoma invasion and metastasis. *Cancer Res* 66:8319–8326
36. Tarin D (2005) The fallacy of epithelial mesenchymal transition in neoplasia. *Cancer Res* 65: 5996–6000
37. Tarin D (2005) Carcinoma invasion and metastasis: A role for epithelial–mesenchymal transition? Response. *Cancer Res* 65:5995–5995
38. Lin X, Tomblor E, Nelson PJ et al (1996) A novel src- and ras-suppressed protein kinase C substrate associated with cytoskeletal architecture. *J Biol Chem* 271:28430–28438
39. Gelman IH, Lee K, Tomblor E et al (1998) Control of cytoskeletal architecture by the src-suppressed C kinase substrate, SSeCKS. *Cell Motil Cytoskeleton* 41:1–17
40. Xia W, Unger P, Miller L et al (2001) The *Src*-suppressed C kinase substrate, SSeCKS, is a potential metastasis inhibitor in prostate cancer. *Cancer Res* 61:5644–5651
41. Lin X, Nelson P, Gelman IH (2000) SSeCKS, a major protein kinase C substrate with tumor suppressor activity, regulates G (1)→S progression by controlling the expression and cellular compartmentalization of cyclin D. *Mol Cell Biol* 20:7259–7272
42. Xia W, Gelman IH (2002) Mitogen-induced, FAK-dependent tyrosine phosphorylation of the SSeCKS scaffolding protein. *Exp Cell Res* 277:139–151
43. Steeg PS, Bevilacqua G, Kopper L et al (1988) Evidence for a novel gene associated with low tumor metastatic potential. *J Natl Cancer Inst* 80:200–204
44. Pozzatti R, Muschel RJ, Williams J et al (1986) Primary rat embryo cells transformed by one or two oncogenes show different metastatic potentials. *Science* 232:223–227
45. Hartsough MT, Steeg PS (2000) Nm23/nucleoside diphosphate kinase in human cancers. *J Bioenerg Biomembranes* 32:301–308
46. Freije JM, MacDonald NJ, Steeg PS (1998) Nm23 and tumour metastasis: basic and translational advances. *Biochem Soc Symp* 63:261–271
47. McDermott WG, Boissan M, Lacombe ML et al (2007) Nm23-H1 homologs suppress tumor cell motility and anchorage independent growth. *Clin Exp Metastasis*. doi:10.1007/s10585-007-9128-0
48. Hailat N, Keim DR, Melhem RF et al (1991) High levels of p19/nm23 protein in neuroblastoma are associated with advanced stage disease and with *N-myc* gene amplification. *J Clin Invest* 88:341–345
49. Fournier HN, Dupé-Manet S, Bouvard D et al (2002) Integrin cytoplasmic domain-associated protein 1α (ICAP-1α) interacts directly with the metastasis suppressor nm23-H2, and both

- proteins are targeted to newly formed cell adhesion sites upon integrin engagement. *J Biol Chem* 277:20895–20902
50. Tee YT, Chen GD, Lin LY et al (2006) Nm23-H1: a metastasis-associated gene. *Taiwan J Obstet Gynecol* 45:107–113
 51. Otsuki Y, Tanaka M, Yoshii S et al (2001) Tumor metastasis suppressor nm23H1 regulates Rac1 GTPase by interaction with Tiam1. *Proc Natl Acad Sci* 98:4385–4390
 52. Kuppens DA, Lan K, Knight JS et al (2005) Regulation of matrix metalloproteinase 9 expression by Epstein-Barr virus nuclear antigen 3C and the suppressor of metastasis Nm23-H1. *J Virol* 79:9714–9724
 53. Che G, Chen J, Liu L et al (2006) Transfection of nm23-H1 increased expression of beta-Catenin, E-Cadherin and TIMP-1 and decreased the expression of MMP-2, CD44v6 and VEGF and inhibited the metastatic potential of human non-small cell lung cancer cell line L9981. *Neoplasma* 53:530–537
 54. Horak CE, Lee JH, Elkahoun AG et al (2007) Nm23-H1 suppresses tumor cell motility by down-regulating the lysophosphatidic acid receptor EDG2. *Cancer Res* 67:7238–7246
 55. Kishi J, Tanaka R, Koiwai O et al (1994) Gelatinases and metalloproteinase inhibitor secreted by murine colonic carcinoma cells with differing metastatic potential. *Cell Biol Int* 18:165–170
 56. Li H, Fang W, Shi Z (1997) Effects of TIMP-2 gene transfection on biological behaviors of a metastatic human lung carcinoma cell line. *Zhonghua Yi Xue Za Zhi* 77:652–656
 57. Lopez-Otin C, Matrisian LM (2007) Emerging roles of proteases in tumour suppression. *Nat Rev Cancer* 7:800–808
 58. Takahashi C, Sheng Z, Horan TP et al (1998) Regulation of matrix metalloproteinase-9 and inhibition of tumor invasion by the membrane-anchored glycoprotein RECK. *Proc Natl Acad Sci* 95:13221–13226
 59. Noda M, Oh J, Takahashi R et al (2003) RECK: a novel suppressor of malignancy linking oncogenic signaling to extracellular matrix remodeling. *Cancer Metastasis Rev* 22:167–175
 60. Correa TCS, Brohem CA, Winnischofer SMB et al (2006) Downregulation of the RECK-tumor and metastasis suppressor gene in glioma invasiveness. *J Cell Biochem* 99:156–167
 61. Span PN, Sweep CGJF, Manders P et al (2003) Matrix metalloproteinase inhibitor reversion-inducing cysteine-rich protein with Kazal motifs – A prognostic marker for good clinical outcome in human breast carcinoma. *Cancer* 97:2710–2715
 62. Oh J, Seo DW, Diaz T et al (2004) Tissue inhibitors of metalloproteinase 2 inhibits endothelial cell migration through increased expression of RECK. *Cancer Res* 64:9062–9069
 63. Noda M, Takahashi C (2007) Recklessness as a hallmark of aggressive cancer. *Cancer Sci* 98:1659–1665
 64. Chang HC, Cho CY, Hung WC (2006) Silencing of the metastasis suppressor RECK by RAS oncogene is mediated by DNA methyltransferase 3b-induced promoter methylation. *Cancer Res* 66:8413–8420
 65. Chang HC, Liu LT, Hung WC (2004) Involvement of histone deacetylation in ras-induced down-regulation of the metastasis suppressor RECK. *Cell Signal* 16:675–679
 66. Liu LT, Chang HC, Chiang LC et al (2003) Histone deacetylase inhibitor up-regulates RECK to inhibit MMP-2 activation and cancer cell invasion. *Cancer Res* 63:3069–3072
 67. Oh J, Takahashi R, Kondo S et al (2001) The membrane-anchored MMP inhibitor RECK is a key regulator of extracellular matrix integrity and angiogenesis. *Cell* 107:789–800
 68. Li SL, Gao DL, Zhao ZH et al (2007) Correlation of matrix metalloproteinase suppressor genes RECK, VEGF, and CD105 with angiogenesis and biological behavior in esophageal squamous cell carcinoma. *World J Gastroenterol* 13:6076–6081
 69. Dong JT, Lamb PW, Rinker-Schaeffer CW et al (1995) KAI1, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2. *Science* 268:884–886
 70. Liu WM, Zhang XA (2006) KAI1/CD82, a tumor metastasis suppressor. *Cancer Lett* 240:183–194
 71. Kauffman EC, Robinson VL, Stadler WM et al (2003) Metastasis suppression: the evolving role of metastasis suppressor genes for regulating cancer cell growth at the secondary site. *J Urol* 169:1122–1133
 72. Jee BK, Park KM, Surendran S et al (2006) KAI1/CD82 suppresses tumor invasion by MMP9 inactivation via TIMP1 up-regulation in the H1299 human lung carcinoma cell line. *Biochem Biophys Res Commun* 342:655–661
 73. Bandyopadhyay S, Zhan R, Chaudhuri A et al (2006) Interaction of KAI1 on tumor cells with DARC on vascular endothelium leads to metastasis suppression. *Nat Med* 12:933–938
 74. Seraj MJ, Samant RS, Verderame MF et al (2000) Functional evidence for a novel human breast carcinoma metastasis suppressor, *BRMS1*, encoded at chromosome 11q13. *Cancer Res* 60:2764–2769
 75. Shevde LA, Samant RS, Goldberg SF et al (2002) Suppression of human melanoma metastasis by the metastasis suppressor gene, *BRMS1*. *Exp Cell Res* 273:229–239
 76. Zhang S, Lin QD, Di W (2006) Suppression of human ovarian carcinoma metastasis by the metastasis-suppressor gene, *BRMS1*. *Int J Gynecol Cancer* 16:522–531
 77. Lombardi G, Di Cristofano C, Capodanno A et al (2006) High level of messenger RNA for *BRMS1* in primary breast carcinomas is associated with poor prognosis. *Int J Cancer* 120:1169–1178
 78. Zhang Z, Yamashita H, Toyama T et al (2006) Reduced expression of the breast cancer metastasis suppressor 1 mRNA is correlated with poor progress in breast cancer. *Clin Cancer Res* 12:6410–6414
 79. Ohta S, Lai EW, Pang ALY et al (2005) Downregulation of metastasis suppressor genes in malignant pheochromocytoma. *Int J Cancer* 114:139–143
 80. Stark AM, Tongers K, Maass N et al (2004) Reduced metastasis-suppressor gene mRNA-expression in breast cancer brain metastases. *J Cancer Res Clin Oncol* 131:191–198
 81. Kelly LM, Buggy Y, Hill A et al (2005) Expression of the breast cancer metastasis suppressor gene, *BRMS1*, in human breast carcinoma: lack of correlation with metastasis to axillary lymph nodes. *Tumor Biol* 26:213–216
 82. Hicks DG, Yoder BJ, Short S et al (2006) Loss of *BRMS1* protein expression predicts reduced disease-free survival in hormone receptor negative and HER2 positive subsets of breast cancer. *Clin Cancer Res* 12:6702–6708
 83. Hurst DR, Mehta A, Moore BP et al (2006) Breast cancer metastasis suppressor 1 (*BRMS1*) is stabilized by the Hsp90 chaperone. *Biochem Biophys Res Commun* 348:1429–1435
 84. Meehan WJ, Samant RS, Hopper JE et al (2004) Breast cancer metastasis suppressor 1 (*BRMS1*) forms complexes with retinoblastoma-binding protein 1 (*RBP1*) and the mSin3 histone deacetylase complex and represses transcription. *J Biol Chem* 279:1562–1569
 85. Champine PJ, Michaelson J, Weimer B et al (2007) Microarray analysis reveals potential mechanisms of *BRMS1*-mediated metastasis suppression. *Clin Exp Metastasis* 24:551–565
 86. Liu Y, Smith PW, Jones DR (2006) Breast cancer metastasis suppressor 1 functions as a corepressor by enhancing histone deacetylase 1-mediated deacetylation of RelA/p65 and promoting apoptosis. *Mol Cell Biol* 26:8683–8696
 87. Phadke PA, Vaidya KS, Nash KT et al (2008) *BRMS1* suppresses breast cancer experimental metastasis to multiple

- organs by inhibiting several steps of the metastatic process. *Am J Pathol* (in press)
88. Simon AM (1999) Gap junctions: more roles and new structural data. *Trends Cell Biol* 9:169–170
 89. Samant RS, Seraj MJ, Saunders MM et al (2001) Analysis of mechanisms underlying *BRMS1* suppression of metastasis. *Clin Exp Metastasis* 18:683–693
 90. Saunders MM, Seraj MJ, Li ZY et al (2001) Breast cancer metastatic potential correlates with a breakdown in homospecific and heterospecific gap junctional intercellular communication. *Cancer Res* 61:1765–1767
 91. Kapoor P, Saunders MM, Li Z et al (2004) Breast cancer metastatic potential: Correlation with increased heterotypic gap junctional intercellular communication between breast cancer cells and osteoblastic cells. *Int J Cancer* 111:693–697
 92. Rinker-Schaeffer CW, Hawkins AL, Ru N et al (1994) Differential suppression of mammary and prostate cancer metastasis by human chromosomes 17 and 11. *Cancer Res* 54:6249–6256
 93. Chekmareva MA, Hollowell CP, Smith RC et al (1997) Localization of prostate cancer metastasis-suppressor activity on human chromosome 17. *Prostate* 33:271–280
 94. Yoshida BA, Dubauskas Z, Chekmareva MA et al (1999) Identification and characterization of candidate prostate cancer metastasis-suppressor genes encoded on human chromosome 17. *Cancer Res* 59:5483–5487
 95. Yoshida BA, Dubauskas Z, Chekmareva MA et al (1999) Mitogen-activated protein kinase kinase 4/stress-activated protein/Erk kinase 1 (*MKK4/SEK1*), a prostate cancer metastasis suppressor gene encoded by human chromosome 17. *Cancer Res* 59:5483–5487
 96. Kim HL, Van der Griend DJ, Yang X et al (2001) Mitogen-activated protein kinase kinase 4 metastasis suppressor gene expression is inversely related to histological pattern in advancing human prostatic cancers. *Cancer Res* 61:2833–2837
 97. Yamada SD, Hickson JA, Hrobowski Y et al (2002) Mitogen-activated protein kinase kinase 4 (*MKK4*) acts as a metastasis suppressor gene in human ovarian carcinoma. *Cancer Res* 62:6717–6723
 98. Hickson JA, Huo D, Vander Griend DJ et al (2006) The p38 Kinases *MKK4* and *MKK6* suppress metastatic colonization in human ovarian carcinoma. *Cancer Res* 66:2264–2270
 99. Vander Griend DJ, Kocherginsky M, Hickson JA et al (2005) Suppression of metastatic colonization by the context-dependent activation of the c-jun NH2-terminal kinase kinases *JNKK1/MKK4* and *MKK7*. *Cancer Res* 65:10984–10991
 100. Vander Griend DJ, Rinker-Schaeffer CW (2004) A new look at an old problem: the survival and organ-specific growth of metastases. *Sci STKE* 2004:216pe3
 101. Aguirre-Ghiso JA (2006) The problem of cancer dormancy – understanding the basic mechanisms and identifying therapeutic opportunities. *Cell Cycle* 5:1740–1743
 102. Aguirre-Ghiso JA, Estrada Y, Liu D et al (2003) ERK(MAPK) activity as a determinant of tumor growth and dormancy; regulation by p38(SAPK). *Cancer Res* 63:1684–1695
 103. Lee J-H, Miele ME, Hicks DJ et al (1996) *KiSS-1*, a novel human malignant melanoma metastasis-suppressor gene. *J Natl Cancer Inst* 88:1731–1737
 104. Nash KT, Welch DR (2006) The *KISS1* metastasis suppressor: mechanistic insights and clinical utility. *Front Biosci* 11:647–659
 105. Nash KT, Phadke PA, Navenot J-M et al (2007) *KISS1* metastasis suppressor secretion, multiple organ metastasis suppression, and maintenance of tumor dormancy. *J Natl Cancer Inst* 99:309–321
 106. Kotani M, Detheux M, Vandenbogaerde A et al (2001) The metastasis suppressor gene *KiSS-1* encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor *GPR54*. *J Biol Chem* 276:34631–34636
 107. Muir AI, Chamberlain L, Elshourbagy NA et al (2001) *AXOR12*: A novel human G protein-coupled receptor, activated by the peptide *KiSS-1*. *J Biol Chem* 276:28969–28975
 108. Ohtaki T, Shintani Y, Honda S et al (2001) Metastasis suppressor gene *KiSS1* encodes peptide ligand of a G-protein-coupled receptor. *Nature* 411:613–617