Metastasis suppressor genes: signal transduction, cross-talk and the potential for modulating the behavior of metastatic cells

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In the past decade, research from various disciplines has stimulated a re-evaluation of our ideas of how cancers metastasize. Two important findings have been fundamental to this re-evaluation: that cancer cells are subject to growth regulation at the secondary site and that a specific class of proteins suppresses the metastatic phenotype. These proteins are encoded by *metastasis suppressor genes*, which are operationally defined as genes that suppress *in vivo* metastasis without inhibiting primary tumor growth when transfected into metastatic cell lines and injected into experimental animals. Recent biochemical studies have shown that certain metastasis suppressor proteins participate in highly conserved signal transduction cascades that mediate cellular responses to growth factors, cytokines and cellular stresses. Further elucidation of the biochemical foundations of these pathways coupled with strong *in vivo* studies should give us insight into the mechanisms of cancer metastasis, and may hold important implications for the future of cancer staging and therapy, using both existing and novel modalities. ***Anti-Cancer Drugs*** **15:559–568**

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Introduction

For the majority of tumors, presence of clinically detectable metastases is the hallmark of incurable disease. The importance of metastasis has been appreciated for more than century [1]. Recent improvements in cancer survival are for the most part the outcome of our ability to detect and treat primary tumors via advances in surgery, radiation oncology and medical oncology [2]. However, these modalities have shown limited efficacy against metastatic disease. During the past 30 years metastasis research has undergone a renaissance, with a flurry of clinical, translational and basic investigations that have illuminated many of the genetic and molecular alterations within the metastatic 'black box' [3]. Such knowledge has unfortunately resulted in few therapeutic advances in the control of metastatic tumor burden.

At the heart of this disconnect between experimental findings and improved clinical outcomes is an inability to develop a coherent mechanistic understanding of metastases. For example, establishing a correlation between 'metastatic ability' and molecular and cellular parameters (e.g. changes in tumor pathology, increased *in vitro* motility, mutation of cancer-related genes or gene expression profiles, etc.) is relatively straightforward. The current challenge resides in conducting the appropriate functional studies that will demonstrate a causal relationship between these specific alterations and metastasis.

This review will discuss the complementary research in the fields of metastasis and signal transduction that has identified signaling pathways controlling metastatic colonization. We will focus on studies of *metastasis suppressor gene* function. These genes encode proteins that specifically suppress metastasis formation while having no discernable effect on primary tumor growth. The mechanisms by which metastasis suppressor proteins suppress colonization and the specific biochemical pathways through which these proteins function will be described. We focus on Nm23, MKK4 and RKIP as examples of this line of study because of their association with well-characterized, and potentially interacting, signaling pathways. We conclude with therapeutic implications of metastasis research, including current efforts to identify substances that modulate the

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expression of metastasis suppressor genes and the function of their encoded proteins.

Biology of metastasis—rethinking the rules of the game

How do we improve treatments for metastatic disease? This is the daunting question faced by all translational metastasis researchers. Conceptually, the answer is straightforward. First, we must observe the complex, natural process of metastasis. These observations are used to develop theories or 'rules of the game' that can be tested experimentally [4,5]. We then try to use this accumulated knowledge to determine how cancer cells metastasize in order to perturb this process therapeutically. Although a variety of approaches have been taken to address this problem, all of the strategies are based on the seminal and far-reaching finding that the development of metastasis is not random [1]. This finding implies that one should be able to predict both the clinical pattern of metastasis for a certain cancer, and discern biochemical and molecular changes responsible for the observed metastases. Once identified, such changes might suggest new treatment strategies.

The process of metastasis requires a complex set of cellular functions, many of which can be initiated by multiple, redundant stimuli. For more than two decades researchers believed that escape of a cell from the primary tumor was the rate-limiting event determining metastatic efficiency [6]. Recently, multiple independent laboratories studying the clinical, biological and molecular determinants of metastasis have clearly shown that disseminated cancer cells at a secondary site remain subject to growth controls. Taken together, these studies show unequivocally that tumor cells readily leave the primary tumor, but often fail to proliferate at an ectopic site [3,7,8]. These findings demonstrate that cells that have acquired the ability to escape from primary tumor, survive in the circulation and reach secondary sites are not by definition autonomous; they remain subject to at least some of the growth controls in their immediate environment. Thus, cellular proliferation at the secondary site, hereafter referred to as *metastatic colonization*, must be considered a potential rate-limiting step of metastasis.

The clinical importance of understanding these new rules resides in our ability to target cells at or before the rate-limiting step. For instance, a drug that inhibits primary tumor invasion will not be clinically useful if, at the time of a cancer diagnosis, a large proportion of primary tumors have already seeded secondary sites. In recent years, experiments utilizing RT-PCR and immunohistochemistry have indicated that tumor cells may disseminate from the primary tumor very early in the course of disease, and often lie dormant at secondary sites as clinically undetectable microscopic metastases at the time of a cancer diagnosis [9–11]. Simultaneously, Chambers and her coworkers have developed novel techniques of intravital video-microscopy and cell accounting which facilitate the utilization of standard metastasis assays to visualize and quantify the steps of metastasis [3]. Their experiments have yielded *in vivo* data from experimental, real-time models which support the findings from clinical studies [12]. Interestingly, biological studies aimed at the identification and characterization of metastasis suppressor genes also identified metastatic colonization as a rate-determining step for formation of overt metastases [13]. For example, *in vivo* metastasis assays demonstrate that MKK4 and KiSS1 expressing metastatic tumor cells successfully arrest in capillary beds at secondary sites in a size dependent manner but fail to proliferate [7,8]. Thus, data from three independent avenues of metastasis research has identified metastatic colonization as an important step in the development of both experimental and clinical metastases.

Metastasis suppressor gene studies

The observation that primary tumor formation is necessary, but not sufficient, for the development of metastasis is a fundamental tenant of metastasis research. The identification of tumor suppressor genes whose loss of function is a critical event in tumorigenesis prompted the hypothesis that a functional loss of specific genetic products could similarly allow the development of metastatic disease [14]. To date, 11 metastasis suppressor genes have been reported, the first being Nm23 which was identified in 1988 (see Table 1) [7,15–23]. There are several excellent comprehensive reviews which discuss the search for metastasis suppressor genes and their potential functions [13,14,24]. A common feature of these studies is the requirement for candidate genes to suppress *in vivo* metastasis in well-characterized models without suppressing tumorigenesis.

Non-metastatic 23 (Nm23)

Steeg *et al.* completed the first published study to test the metastasis suppressor gene hypothesis in 1988. They identified Nm23 as a gene that was down-regulated in highly metastatic murine melanoma cells compared to less metastatic, genetically related counterparts [15]. While reports showing a correlation between decreased Nm23 gene expression and increased metastatic propensity in clinical samples soon followed, determining the biochemical function of Nm23 protein was more problematic [25,26]. Eight Nm23 isotypes have been identified thus far but only Nm23-H1 and Nm23-H2 have been formally tested for metastasis suppressing activity. Because of Nm23's homology to a *Drosophila* counterpart, initial efforts focused on determining how its function as a nucleoside diphosphate kinase suppressed metastasis *in vivo*. However, Nm23 proteins also have functions in addition to that of a nucleoside diphosphate kinase [27].

Table 1 Metastasis suppressor genes

Gene	Cancer cell type suppressed	Current known function
Nm23	melanoma [15], breast [79], colon [80], oral squamous cell [81]	histidine kinase; phosphorylates KSR, which might reduce ERK1/2 activation
MKK4	prostate [7], ovarian [33]	MAPKK; phosphorylates and activates p38 and JNK kinases
KAI1	prostate [16], breast [82]	integrin interaction EGFR desensitization
BRMS1	breast [17], melanoma [83]	gap-junctional communication
KiSS1	melanoma [18], breast [84]	G-protein-coupled receptor ligand
RhoGD12	bladder [19]	regulates RHO and RAC function
CRSP3	melanoma [20]	transcriptional co-activator
VDUP1	melanoma [20]	thioredoxin inhibitor
SSeCKS	prostate [21]	scaffold protein of MAPK/ERK system
RKIP	prostate [22]	inhibits Raf-mediated MEK phosphorylation
Drg-1	prostate [23]	possible downstream mediator of MKK4 [14,48]

Nm23-H2 was implicated as a DNA *trans*-activator of *myc* in 1993 [28]. Furthermore, Nm23 proteins interact with many other proteins, most of which have no known role in metastasis suppression. The most recent and comprehensive mechanistic studies of Nm23 identified an unexpected function for this protein as a histidine protein kinase. Work by Hartsough, *et al.* provide biochemical evidence supporting the hypothesis that metastasis suppression is mediated by Nm23's histidine protein kinase function in relationship to the kinase suppressor of *ras* (Ksr) protein [29]. The potential role of Nm23 and Ksr in signaling cascades regulating metastatic growth will be discussed in the following sections.

Mitogen-activated protein (MAP) kinase kinase 4 (MKK4/JNKK1/SEK1)

Using microcell-mediated chromosomal transfer coupled with positional cloning, our laboratory identified MKK4 (also referred to as JNKK1, SEK2 or MEK4) as a metastasis suppressor gene encoded by human chromosome 17 [7,30,31]. MKK4 is a duel specificity kinase that phosphorylates both threonine and tyrosine residues, and has been shown to phosphorylate the JNK (TPY) and p38 (TGY) MAPK activation motifs. The MKK4 protein is widely expressed throughout human and mouse tissues, and has a role in hepatic, neural and thymic development in mice [32]. Functional and clinical correlative studies identified a role for MKK4 protein in the suppression of metastasis in ovarian and prostate cancers [32–35]. The function of MKK4 in tumor progression and metastasis may be context dependent, as previous studies examining loss of heterozygosity of human chromosome 17 suggest that it may function as a tumor suppressor gene in a small percentage of pancreatic, biliary and breast cancers [36]. The role of MKK4 in gastric cancer is more controversial; reports have shown that MKK4 protein expression is *increased* in invasive gastric cancer, while another study indicated no change in MKK4 protein expression, but significant loss of heterozygosity at telomeric markers for MKK4 [37,38]. Such seemingly contradictory activities illustrate the challenges faced when trying to determine how a metastasis suppressor protein such as MKK4 functions *in vivo* and within a particular cancer type.

Raf kinase inhibitor protein (RKIP)

Fu *et al.* used microarray analysis to identify candidate metastasis suppressor genes that were under-expressed in the metastatic human prostate cancer cell line C4-2B compared to its non-metastatic precursor LNCaP [22]. This analysis identified RKIP as a candidate metastasis suppressor gene. Immunohistochemical studies using a cohort of clinical prostate cancer specimens showed that RKIP levels were highest in normal prostate epithelia, diminished in primary tumors and absent in prostate cancer metastases. Ectopic expression of RKIP in metastatic C4-2 cells reduced metastasis *in vivo* as well as invasion *in vitro* as compared to controls. Furthermore, non-metastatic LNCaP cells acquired metastatic properties when transfected with anti-sense RKIP DNA [22].

KAI1/CD82

KAI1/CD82 encodes a putative membrane protein in the tetraspanin superfamily. Tetraspanins localize to the cell surface where they are believed to act as 'organizers' of large protein complexes regulating adhesion, migration, growth and differentiation. Expression of ectopic KAI1 in AT6.1 Dunning rat prostate cancer cells reduced the number of overt lung metastases by 66% in comparison with parental AT6.1 controls [16]. Clinical data also supports a role for KAI1 in the suppression of prostate cancer metastasis. KAI1 expression is down-regulated in both metastatic and high-grade tumors, as well as in 100% of lymph node metastases [39,40]. KAI1 may act as a general cancer metastasis suppressor, as its down-regulation is seen clinically with the progression to metastasis for cancers of the liver, colon, esophagus, pancreas, lung, bladder (invasive phenotype), ovaries, cervix (grade only) and breast.

The mechanism by which KAI1 suppresses metastases is not clear. KAI1 may function through its interactions with certain membrane proteins implicated in metastasis progression, including E-cadherin, β_1 integrins and the epidermal growth factor receptor (EGFR). Both EGFR and β_1 integrins have been shown to enhance the number of spontaneous metastases of cancer cells in mice. Interestingly, both are implicated specifically in

promoting tumor cell growth at the secondary site [41,42]. While the regulatory effects of KAI1 interactions with E-cadherin and integrins are not known, KAI1 association inhibits EGFR signaling *in vitro*, suggesting a potential *in vivo* function.

KiSS1

Like MKK4, KiSS1 appears to suppress metastatic colonization. Using fluorescent tagging, researchers found that metastatic melanoma cells suppressed by KiSS1/chromosome 6 were still able to reach the target organ tissue, present as both single cells and clusters of less than 10 cells [8]. Extending the duration of the metastasis assay did not lead to outgrowth of these cells, confirming that they were growth inhibited. KiSS1 was ultimately mapped to chromosome 1q, a finding which led to the hypothesis that a KiSS1 regulatory element resides on chromosome 6 (see CRSP3, below) [18]. The mechanism by which KiSS1 regulates cell growth has not been confirmed, but is likely related to its association with a G-protein-coupled membrane receptor (hOT7T175/AXOR12) [43]. Binding of the KiSS1 protein to this receptor *in vitro* induces rapid changes in cell adhesion and actin organization, although the functional significance of these changes has not been precisely defined [44]. In melanomas, loss of protein expression correlated with progression to metastasis, supporting a role for the KiSS1 gene in the metastasis suppression of clinical disease [45].

Cofactor required for specificity protein (SP) activation 3 (CRSP3)

An activator of the SP1, CRSP3 may play a role in the regulation of KISS1 expression [20]. The CRSP3 gene lies on a region of chromosome 6 which suppresses metastasis when introduced into metastatic cells by microcell-mediated chromosome transfer. To date, no studies have been published defining the role of CRSP3 clinically.

Breast cancer metastasis suppressor 1 (BRMS1)

In melanoma and breast cancer cells, expression of BRMS1 appears to restore normal gap junction phenotype, suggesting a role in cell–cell communication. This effect may be significant in metastasis regulation, as alteration in gap junction phenotype may be important in the clinical progression of several cancers [46]. Mechanisms by which loss of normal gap junction phenotype may promote metastasis have been proposed based on *in vitro* studies, and include reduced communication between cells within the primary tumor and enhanced 'seed-and-soil' communication at the secondary site. The latter possibility may involve inhibition of disseminated tumor cell growth as gap junctions have well established roles in negative growth control. Studies investigating the role of BRMS1 in additional cancer types and clinical disease are currently underway.

Differentiation-related gene 1 (Drg-1)

Drg-1 was initially identified as a metastasis suppressor gene in colon adenocarcinoma by Guan *et al.* by comparing gene expression in metastatic SW620 colon cancer cells compared to non-metastatic SW480 cells [23]. Transfection of metastatic colon adenocarcinoma lines with Drg-1 induced morphological and molecular indicators of increased epithelial cell differentiation. Recently, Bandyopadhyay *et al.* showed Drg-1 to have metastasis-suppressing activity in human prostate cancer cells and that its expression in clinical samples varies inversely with Gleason grading [47]. The mechanism by which the Drg-1 protein functions to suppress metastasis is unknown, although it may interact with JNK/p38 pathways [48].

Rho GDP dissociation inhibitor 2 (RhoGDI2)

RhoGDIs stabilize the inactive GDP-bound state of the Rho GTPases. RhoGDI2 was identified as a putative metastasis suppressor in human bladder carcinomas by Gildea *et al.* [19]. RhoGDI2 effectively blocks activity by the Rho and Rac GTPases, which have been implicated in cytoskeletal reorganizations at the cell surface that mediate invasion and cell-cell interactions [49]. Rho, Rac and RhoGDI2 also may participate in p38, JNK and ER pathways [50].

The Src-suppressed C kinase substrate (SSeCKS)

SSeCKS probably functions as a scaffolding protein for protein kinase C (PKC)- and protein kinase A (PKA)-dependent signaling complexes [51]. The human ortholog Gravin was mapped by Xia *et al.* to a region on human chromosome 6 that is often deleted in advanced prostate cancers [21]. They found that Gravin expression was decreased in highly metastatic prostate cancer lines compared to less aggressive cells, and that transfecting metastatic cells with SSeCKS decreased anchorage-independent growth *in vitro* and a decrease in lung metastases in spontaneous metastasis assays. The precise mechanism by which SSeCKS suppresses metastasis is currently under investigation.

Signal transduction, cross-talk and metastatic behavior

The concept that aggressive cancer cells replicate autonomously and thwart growth control mechanisms irrespective of their environment has been challenged by clinical and experimental studies of metastasis. Although these cells often have an aberrant response to the extracellular signals that mediate growth and differentiation, it is a response nonetheless. This finding is important because it implies that intracellular signaling has been perturbed in a distinct way that permits metastatic cells to elude growth control mechanisms at the secondary site. Understanding how these signaling events are distorted requires researchers to design meaningful mechanistic studies of metastasis

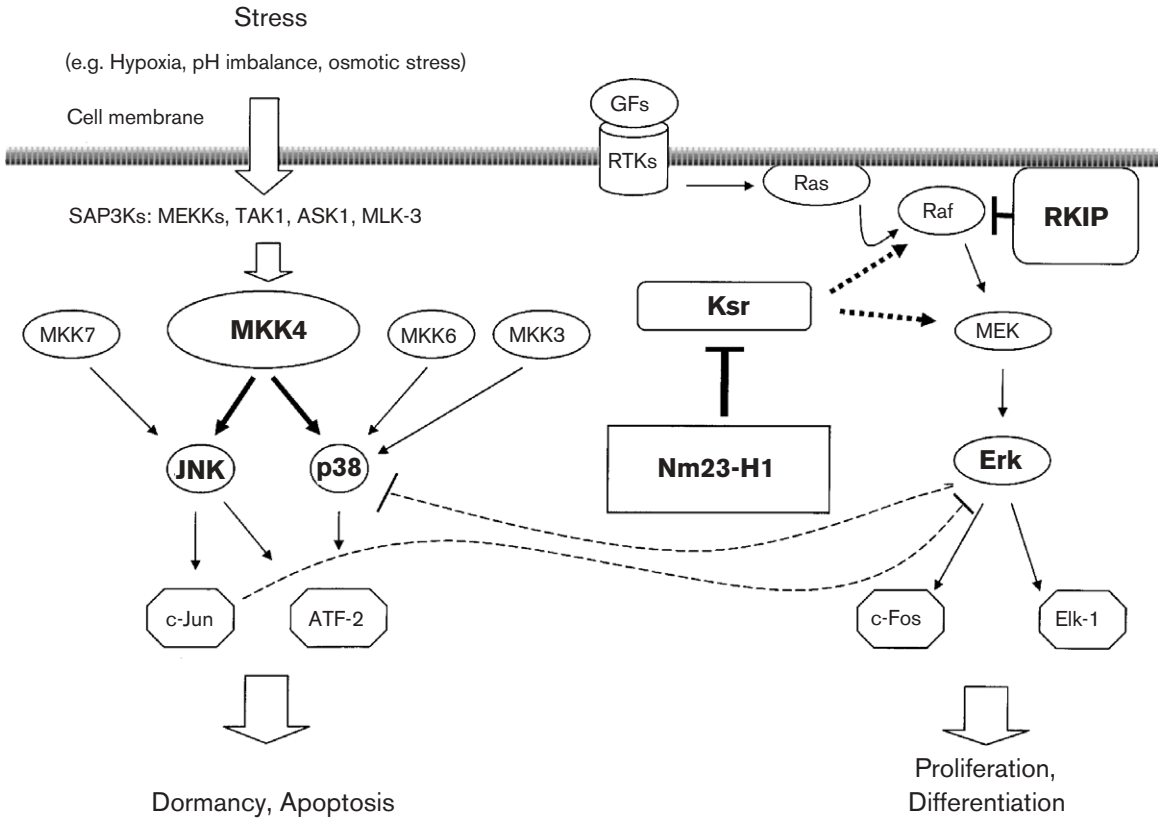
suppression. A wealth of biologic studies have shown that the microenvironment of the metastatic site often provides extracellular signals that are distinct from those at the primary tumor [52,53]. Thus, metastatic growth represents new responses to new signals. Clearly, the interaction between tumor cells and their microenvironment must be considered in the design of studies to determine metastasis suppressor protein function *in vivo*.

Biochemical data currently support a model in which Nm23, RKIP and MKK4 proteins participate in conserved eukaryotic signal transduction pathways. These signaling cascades are comprised of three parallel modules: the mitogen-activated protein kinase/extracellular signal regulated kinase (MAPK/ERK) module, the stress-activated protein kinase/c-Jun N-terminal Kinase (SAPK/JNK) and the p38 module (see Fig. 1). These phenotypically distinct, but biochemically interconnected, signaling modules relay extracellular stimuli through protein kinase cascades that activate transcription factors and affect cellular responses to stress, inflammation and growth factors [54,55].

The MAPK/ERK pathway involves the receptor tyrosine kinase (RTK) class of transmembrane proteins and serine-threonine kinases (e.g. PDGF, EGF, insulin receptors, etc.). Typically, cell surface receptors interact with adaptor molecules to recruit a protein kinase complex to the cell membrane. The prototypical MAPK/ERK pathway involves PTKs that interact with Ras through proteins with SH2 and SH3 adaptor domains. In its active form, Ras recruits Raf, a MAP kinase kinase kinase (MAP3K), to the membrane complex resulting in the activation of a target MAP/ERK Kinase (MEK). Phosphorylation of MEK results in the activation and subsequent translocation of ERK to the nucleus, where it phosphorylates various transcription factors (e.g. Elk-1, c-Fos) and DNA-binding proteins [54,55].

Signal amplification and regulation may occur at each step in this pathway. Specificity of the signal is conferred through both the affinity of a target kinase for a given target as well as protein expression levels. Additional regulation is conferred by interaction with other scaffolding or adaptor proteins. In the case of metastasis suppression, the Nm23-H1 protein interacts with the MAPK module by binding to and phosphorylating the Kinase Suppressor of Ras (KSR) protein, a scaffold protein that has role in the regulation of the MAPK/ERK pathway upstream of MEK [56]. Ksr has been shown

Fig. 1



Schematic diagram of signaling pathways regulated by metastasis suppressor proteins. Proteins of interest are in bold.

to positively regulate Ras-mediated MAPK signaling, suggesting that Nm23's metastasis suppression activity may be mediated by inhibition of Ksr-enabled MAPK signaling [57].

Similarly, RKIP functions as a negative upstream regulator of MAPK/ERK signaling. Raf and MEK interact with RKIP at overlapping sites, and binding of either molecule inhibits binding of the other. Both binding domains must be destroyed in order to alleviate RKIP-mediated MAPK inhibition. Furthermore, studies with constitutively active MEK-DD mutants suggest that RKIP functions upstream of MEK [58]. Thus, RKIP may suppress metastasis by inhibiting Raf-mediated phosphorylation of MEK.

The SAPK/JNK and p38 pathways operate in parallel to MAPK/ERK pathway. In contrast to the association of ERK with proliferation, JNK and p38 have been classically associated with cell cycle arrest and apoptosis in response to environmental stresses and cytokines, pH changes, UV irradiation, hypoxia, and growth factor deprivation [59]. Several MAP2Ks specifically activate either p38- or JNK-mediated signaling. Interestingly MKK4, a duel-specificity kinase with both tyrosine and serine-threonine kinase activities, can phosphorylate and activate both JNK and p38. We are currently working to determine which arm of the cascade MKK4 signals through to suppress metastasis. In addition, the biological mechanism of context-dependent suppression (e.g. apoptosis, quiescence, decreased proliferation, etc.) is also being examined.

While the ERK and SAPK pathways have historically been considered as separate, opposing cellular functions, there is growing evidence that supports various interactions between these pathways [60,61]. In addition to biochemical studies, recent *in vivo* studies suggest an antagonistic relationship between ERK and p38 signal transduction pathways that may determine whether a tumor cell proliferates or enters a state of cell-cycle arrest [60,62]. Aguirre-Ghiso *et al.* demonstrated that the ERK/p38 activity ratio in certain head and neck cancer cell lines correlates with the balance between cellular proliferation and dormancy. In their well-characterized model, persistent ERK activity is maintained through urokinase plasminogen activator receptor (uPAR) activity and its interactions with $\alpha_5\beta_1$ integrin. Their model predicts that shifting the ratio between phospho-ERK and phospho-p38 determines whether a cell will proliferate or remain dormant [62]. In subsequent studies they showed that modulation of the ERK/p38 ratio may induce dormant (high p38) or proliferative (high ERK) phenotypes in additional models (e.g. cancers of the breast, prostate, skin and connective tissue) [60].

Potential clinical applications

The identification of specific signaling pathways that regulate metastatic growth is an important advance, yet there are significant roadblocks to successful incorporation of this knowledge into clinical practice. First of all, the individual metastasis suppressor protein must be considered in terms of the pathway it participates in. Simply turning 'on' or 'off' a single gene (protein) in a signal transduction pathway will inevitably have broader affects than intended, for each pathway may affect, positively or negatively, the regulation of many as yet undocumented processes. Furthermore, the context-specific nature of signal transduction may limit our ability to generalize the affects of modulating a specific pathway to other model systems or tissue types. For instance, although the MKK4-mediated activation of JNK/p38 has an established correlation with metastasis suppression in prostate and ovarian carcinomas, the activation of this same pathway may have a role in the malignant transformation of small cell lung carcinomas [34]. Thus, the effect of a specific signaling pathway may have to be determined on a case-by-case basis.

Metastasis suppressors and adjuvant therapy

In vivo models suggest that metastatic colonization, which as noted is the development of overt metastatic lesions from microscopic deposits, represents a potentially key rate-limiting step of metastatic development. The identification of a role for several of the known metastasis suppressor proteins in the regulation of this process through highly conserved signal transduction pathways represents a significant maturation in our ideas about metastasis. In order to translate these findings into tangible clinical benefit, we must first determine whether the findings from *in vivo* models correlate with the behavior of human cancers. There is an accumulation of clinical data supporting colonization as the critical event in the development of human metastases. Historically, surgical oncologists have theorized that the removal of solid tumors releases tumor cells into the systemic circulation [63–66]. Recent developments in molecular biology have confirmed that cancer cells can readily be found in the blood. Several studies using PCR have shown that cancer cells are often present in the circulation at the time of diagnosis or surgery [9]. As many as 25% of patients undergoing radical retropubic prostatectomy have evidence of hematogenous dissemination of tumor cells during surgery [67]. Immunohistochemical studies have confirmed that solid tumor cells may reside as clinically occult microscopic metastases in bone marrow or other tissues at the time of diagnosis [10,11]. Collectively, these data imply that tumor cells readily escape from human primary tumors, yet fail to colonize a secondary site.

If metastatic colonization represents the rate-limiting step for human cancers, than our knowledge of metastasis

suppressor genes could be used to guide clinical decision-making. Specifically, physicians typically treat patients with adjuvant regimens in order to eliminate microscopic metastatic disease before its progression into clinically detectable disease. The selection of patients for such regimens assumes that microscopic lesions will inevitably grow into clinically significant tumors. Basic metastasis research, however, suggests that a microscopic metastasis will proliferate at the secondary site only if it has undergone specific genetic and epigenetic changes that are distinct from the events of primary tumorigenesis. Disseminated tumor cells that do not possess this ability are not a clinical threat and need not be treated. Clinicians thus subject many patients to the cost and morbidity of adjuvant therapy to achieve clinical benefit in only a few patients who have disease with metastatic capability.

The identification of metastasis suppressor genes/proteins may provide an avenue by which clinicians can interrogate cancer cells for the ability to form clinically relevant metastatic disease. A molecular assay for metastatic ability would allow clinicians to stratify patients into high and low risk for metastatic disease. Such an assay would analyze the expression of the relevant tumor type-specific metastasis suppressor proteins. Clinicians and patients could then choose together whether aggressive adjuvant therapy is warranted.

Of course, significant problems need to be addressed before such an algorithm can be considered viable. First of all, we need to determine which tissue specimens to assay. A logical first choice would be primary tumor samples obtained from biopsies or surgical therapy. However, if our contention that metastasis requires molecular changes distinct from tumorigenesis is valid, than we cannot assume that the genetic profile of primary tumor cells reliably reflects that of disseminated cells. Furthermore, if only a subset of primary tumor cells have the ability to metastasize, will an assay done on a biopsy or tissue specimen necessarily contain the cells of interest?

Such questions lead us to the possibility of examining disseminated tumor cells directly. Bone marrow or other secondary site-directed biopsies combined with methodologies for appropriate cell enrichment can provide samples of disseminated disease for assay [68]. However, invasive techniques introduce additional morbidity. Furthermore, it has not been shown that the genetic profile of disseminated tumor cells is stable over time. A patient that is stratified as low risk after a metastasis suppressor expression assay may need to be re-assayed at some time in the future in order to reconfirm the status of their disease.

A foreseeable strategy might involve the assay of primary tumor cells at the time of surgery. If metastatic capability is present, than adjuvant therapy would be recommended. If no metastatic capability is identified, than the clinician might recommend a search for disseminated cells in the bone marrow or other tumor type-specific locations. These cells could then be assayed either once or repeatedly to determine metastatic ability and guide therapy.

Metastasis suppressor genes and novel cancer therapies

The greatest hope among metastasis researchers is that an understanding of the mechanisms of metastasis suppression may lead to targeted therapies. Tumor metastasis represents the most feared, least treatable and ultimately most lethal consequence of malignancy. Metastatic disease often relegates patients to palliative therapy and decreased quality of life. Some of the remarkable successes in cancer drug design in the last several decades have come from an understanding of the molecular biology of disease. Selective estrogen receptor modulators (SERMs) have become a cornerstone of adjuvant therapy for breast cancer, and are beginning to find a role in breast cancer prophylaxis in high-risk women [69]. The phenomenal success of the tyrosine kinase inhibitor imatinib (Gleevec) in certain patients with chronic myelogenous leukemia and gastrointestinal stromal tumors is another example of how an understanding of the biology of cancer can stimulate the development of novel therapies. Ideally, basic science research in the field of metastasis suppressor genes will similarly translate into clinical therapeutics.

Studies indicate that the majority of metastasis suppressors are down-regulated, but not mutated or deleted, in metastatic lesions [35,39]. This observation may provide a unique therapeutic opportunity because, unlike mutated oncogenes or tumor suppressor genes, exogenous DNA or a constitutively active gene product does not necessarily need to be introduced into malignant cells. Rather, it may be possible to identify compounds that restore expression of silenced metastasis suppressor genes and thus modulate important signal transduction cascades such as the MAPK and SAPK pathways to inhibit metastatic colonization. Alternatively, we may be able to manipulate the signaling pathways in which metastasis suppressor proteins participate without specifically targeting metastasis suppressor proteins.

Preliminary efforts to identify compounds that have a negative affect on metastasis growth through metastasis suppressor pathways are in progress. Currently, drugs that affect metastasis suppressor up-regulation have non-specific, pleiotropic affects on gene expression, and none can be considered a specific activator of a metastasis

Table 2 Compounds that affect metastasis suppressor gene expression

Nm23	anti-inflammatory drugs	indomethacin [70,71]	up-regulates Nm23 expression in breast lines induces alterations in membrane phospholipid profiles
		aspirin [72]	up-regulates Nm23 expression in colon adenocarcinoma cells associated with decreased metastatic phenotype *in vitro*
	hormone exposure	estradiol [73]	up-regulates Nm23-H1 in $ER\alpha^+$ breast cell lines Inhibits invasion *in vitro*
		ATRA [74]	up-regulates Nm23 in hepatocarcinoma cells increased adhesion to ECM *in vitro* decreased chemotaxis *in vitro*
	structural DNA manipulation	5-Aza-CdR [75]	inhibits DNA methylation associated with NM23 up-regulation in breast cell lines decreased metastatic phenotype *in vitro*
		trichostatin A [76]	inhibits histone deacetylase-1 up-regulates Nm23 in gastric cell lines
MKK4	immunomodulation	anti-death receptor antibodies [77]	induce apoptosis *in vitro* activate MKK4/JNK/p38 pathways
		bisindolylmeleimide VIII [78]	enhances affects of anti-death receptor antibodies

suppressor pathway [70–78]. Such compounds include DNA methylation inhibitors, histone deacetylase inhibitors, steroid hormones, anti-inflammatory drugs and immunomodulators (see Table 2). While not specific activators of metastasis suppressor genes, such compounds may yet hold promise in the clinic and are currently being evaluated for *in vivo* metastasis suppressing activity [24].

The development of drugs that specifically target metastatic colonization may come as the functional studies describing the signal transduction pathways regulating metastatic growth are completed. Undoubtedly, there are numerous proteins that interact with SAPK, p38 and ERK pathways, each of which may have potential as a therapeutic target. Perhaps the ideal molecular targets are not the metastasis suppressor genes or proteins themselves, but their antagonists. Identifying a specific inhibitor of a functional metastasis suppressor antagonist could modulate signaling in a therapeutically beneficial manner without accomplishing the clinically difficult task of up-regulating metastasis suppressor gene expression.

Conclusion

Metastasis research has resulted in a re-evaluation of our concepts of growth regulation and signaling in cancer cells. As researchers gain a greater understanding of the biochemical mechanisms of metastasis, we will undoubtedly continue to encounter unanticipated findings that challenge our ideas about malignancy. Hopefully, the further demystification of the metastatic process will facilitate the development of viable new strategies for cancer prognosis and therapy.

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References

1 Paget S. The distribution of secondary growths in cancer of the breast. *Cancer Metastasis Rev* 1989; **8**:98–101.
2 Jemal A, Thomas A, Murray T, Thun M. Cancer statistics [Comment] [Erratum appears in *CA Cancer J Clin* 2002; 52:119]. *CA Cancer J Clin* 2002; **52**:23–47.
3 MacDonald IC, Groom AC, Chambers AF. Cancer spread and micrometastasis development: quantitative approaches for *in vivo* models. *BioEssays* 2002; **24**:885–893.
4 Feynman RP. *Six Easy Pieces: Essentials of Physics, Explained by its Most Brilliant Teacher.* Reading, MA: Helix Books; 1995, p. 145.
5 Hahn WC, Weinberg RA. Rules for making human tumor cells. *N Engl J Med* 2002; **347**:1593–1603.
6 Hall SJ, Thompson TC. Spontaneous but not experimental metastatic activities differentiate primary tumor-derived vs metastasis-derived mouse prostate cancer cell lines. *Clin Exp Metastasis* 1997; **15**:630–638.
7 Yoshida BA, Dubauskas Z, Chekmareva MA, Christiano TR, Stadler WM, Rinker-Schaeffer CW. 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* 1999; **59**:5483–5487.
8 Goldberg SF, Harms JF, Quon K, Welch DR. Metastasis-suppressed C8161 melanoma cells arrest in lung but fail to proliferate. *Clin Exp Metastasis* 1999; **17**:601–607.
9 Raj GV, Moreno JG, Gomella LG. Utilization of polymerase chain reaction technology in the detection of solid tumors [Comment]. *Cancer* 1998; **82**:1419–1442.
10 Cher ML, de Oliveira JG, Beaman AA, Nemeth JA, Hussain M, Wood Jr DP. Cellular proliferation and prevalence of micrometastatic cells in the bone marrow of patients with clinically localized prostate cancer. *Clin Cancer Res* 1999; **5**:2421–2425.
11 Melchior SW, Corey E, Ellis WJ, Ross AA, Layton TJ, Oswin MM, *et al.* Early tumor cell dissemination in patients with clinically localized carcinoma of the prostate. *Clin Cancer Res* 1997; **3**:249–256.
12 Cameron MD, Schmidt EE, Kerkvliet N, Nadkarni KV, Morris VL, Groom AC, *et al.* Temporal progression of metastasis in lung: cell survival, dormancy, and location dependence of metastatic inefficiency. *Cancer Res* 2000; **60**:2541–2546.
13 Kauffman EC, Robinson VL, Stadler WM, Sokoloff MH, Rinker-Schaeffer CW. Metastasis suppression: the evolving role of metastasis suppressor genes for regulating cancer cell growth at the secondary site [Comment]. *J Urol* 2003; **169**:1122–1133.
14 Shevde LA, Welch DR. Metastasis suppressor pathways—an evolving paradigm. *Cancer Lett* 2003; **198**:1–20.
15 Steeg PS, Bevilacqua G, Kopper L, Thorgeirsson UP, Talmadge JE, Liotta LA, *et al.* Evidence for a novel gene associated with low tumor metastatic potential. *J Natl Cancer Inst* 1988; **80**:200–204.
16 Dong JT, Lamb PW, Rinker-Schaeffer CW, Vukanovic J, Ichikawa T, Isaacs JT, *et al.* KAI1, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2 [Comment]. *Science* 1995; **268**:884–886.

17 Seraj MJ, Samant RS, Verderame MF, Welch DR. Functional evidence for a novel human breast carcinoma metastasis suppressor, BRMS1, encoded at chromosome 11q13. *Cancer Res* 2000; **60**:2764–2769.

18 Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, *et al.* KiSS-1, a novel human malignant melanoma metastasis-suppressor gene [Comment] [Erratum appears in *J Natl Cancer Inst* 1997; **89**:1549]. *J Natl Cancer Inst* 1996; **88**:1731–1737.

19 Gildea JJ, Seraj MJ, Oxford G, Harding MA, Hampton GM, Moskaluk CA, *et al.* RhoGDI2 is an invasion and metastasis suppressor gene in human cancer. *Cancer Res* 2002; **62**:6418–6423.

20 Goldberg SF, Miele ME, Hatta N, Takata M, Paquette-Straub C, Freedman LP, *et al.* Melanoma metastasis suppression by chromosome 6: evidence for a pathway regulated by CRSP3 and TXNIP. *Cancer Res* 2003; **63**: 432–440.

21 Xia W, Unger P, Miller L, Nelson J, Gelman IH. The Src-suppressed C kinase substrate, SSeCKS, is a potential metastasis inhibitor in prostate cancer. *Cancer Res* 2001; **61**:5644–5651.

22 Fu Z, Smith PC, Zhang L, Rubin MA, Dunn RL, Yao Z, *et al.* Effects of raf kinase inhibitor protein expression on suppression of prostate cancer metastasis [Comment]. *J Natl Cancer Inst* 2003; **95**:878–889.

23 Guan RJ, Ford HL, Fu Y, Li Y, Shaw LM, Pardee AB. Drg-1 as a differentiation-related, putative metastatic suppressor gene in human colon cancer. *Cancer Res* 2000; **60**:749–755.

24 Steeg PS, Ouatas T, Halverson D, Palmieri D, Salerno M. Metastasis suppressor genes: basic biology and potential clinical use. *Clin Breast Cancer* 2003; **4**:51–62.

25 Bevilacqua G, Sobel ME, Liotta LA, Steeg PS. Association of low nm23 RNA levels in human primary infiltrating ductal breast carcinomas with lymph node involvement and other histopathological indicators of high metastatic potential. *Cancer Res* 1989; **49**:5185–5190.

26 Salerno M, Ouatas T, Palmieri D, Steeg PS. Inhibition of signal transduction by the nm23 metastasis suppressor: possible mechanisms. *Clin Exp Metastasis* 2003; **20**:3–10.

27 Roymans D, Willems R, Van Blockstaele DR, Slegers H. Nucleoside diphosphate kinase (NDPK/NM23) and the waltz with multiple partners: possible consequences in tumor metastasis. *Clin Exp Metastasis* 2002; **19**:465–476.

28 Postel EH, Berberich SJ, Flint SJ, Ferrone CA. Human c-*myc* transcription factor PuF identified as nm23-H2 nucleoside diphosphate kinase, a candidate suppressor of tumor metastasis [Comment]. *Science* 1993; **261**:478–480.

29 Hartsough MT, Morrison DK, Salerno M, Palmieri D, Ouatas T, Mair M, *et al.* Nm23-H1 metastasis suppressor phosphorylation of kinase suppressor of Ras via a histidine protein kinase pathway. *J Biol Chem* 2002; **277**: 32389–32399.

30 Chekmareva MA, Hollowell CM, Smith RC, Davis EM, LeBeau MM, Rinker-Schaeffer CW. Localization of prostate cancer metastasis-suppressor activity on human chromosome 17. *Prostate* 1997; **33**:271–280.

31 Sanchez I, Hughes RT, Mayer BJ, Yee K, Woodgett JR, Avruch J, *et al.* Role of SAPK/ERK kinase-1 in the stress-activated pathway regulating transcription factor c-Jun. *Nature* 1994; **372**:794–798.

32 Cuenda A. Mitogen-activated protein kinase kinase 4 (MKK4). *Int J Biochem Cell Biol* 2000; **32**:581–587.

33 Yamada SD, Hickson JA, Hrobowski Y, Vander Griend DJ, Benson D, Montag A, *et al.* Mitogen-activated protein kinase kinase 4 (MKK4) acts as a metastasis suppressor gene in human ovarian carcinoma. *Cancer Res* 2002; **62**:6717–6723.

34 Xiao L, Lang W. A dominant role for the c-Jun NH_2-terminal kinase in oncogenic *ras*-induced morphologic transformation of human lung carcinoma cells. *Cancer Res* 2000; **60**:400–408.

35 Kim HL, Vander Griend DJ, Yang X, Benson DA, Dubauskas Z, Yoshida BA, *et al.* Mitogen-activated protein kinase kinase 4 metastasis suppressor gene expression is inversely related to histological pattern in advancing human prostatic cancers. *Cancer Res* 2001; **61**:2833–2837.

36 Su GH, Hilgers W, Shekher MC, Tang DJ, Yeo CJ, Hruban RH, *et al.* Alterations in pancreatic, biliary, and breast carcinomas support MKK4 as a genetically targeted tumor suppressor gene. *Cancer Res* 1998; **58**: 2339–2342.

37 Wu CW, Li AF, Chi CW, Huang CL, Shen KH, Liu WY, *et al.* Human gastric cancer kinase profile and prognostic significance of MKK4 kinase. *Am J Pathol* 2000; **156**:2007–2015.

38 Chae KS, Ryu BK, Lee MG, Byun DS, Chi SG. Expression and mutation analyses of MKK4, a candidate tumour suppressor gene encoded by chromosome 17p, in human gastric adenocarcinoma. *Eur J Cancer* 2002; **38**:2048–2057.

39 Dong JT, Suzuki H, Pin SS, Bova GS, Schalken JA, Isaacs WB, *et al.* Down-regulation of the KAI1 metastasis suppressor gene during the progression of human prostatic cancer infrequently involves gene mutation or allelic loss. *Cancer Res* 1996; **56**:4387–4390.

40 Ueda T, Ichikawa T, Tamaru J, Mikata A, Akakura K, Akimoto S, *et al.* Expression of the KAI1 protein in benign prostatic hyperplasia and prostate cancer. *Am J Pathol* 1996; **149**:1435–1440.

41 Morris VL, Schmidt EE, Koop S, MacDonald IC, Grattan M, Khokha R, *et al.* Effects of the disintegrin eristostatin on individual steps of hematogenous metastasis. *Exp Cell Res* 1995; **219**:571–578.

42 Herynk MH, Radinsky R. The coordinated functional expression of epidermal growth factor receptor and c-Met in colorectal carcinoma metastasis. *In Vivo* 2000; **14**:587–596.

43 Stafford LJ, Xia C, Ma W, Cai Y, Liu M. Identification and characterization of mouse metastasis-suppressor KiSS1 and its G-protein-coupled receptor. *Cancer Res* 2002; **62**:5399–5404.

44 Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, *et al.* Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature* 2001; **411**:613–617.

45 Shirasaki F, Takata M, Hatta N, Takehara K. Loss of expression of the metastasis suppressor gene KiSS1 during melanoma progression and its association with LOH of chromosome 6q16.3–q23. *Cancer Res* 2001; **61**:7422–7425.

46 Habermann H, Ray V, Habermann W, Prins GS. Alterations in gap junction protein expression in human benign prostatic hyperplasia and prostate cancer. *J Urol* 2002; **167**:655–660.

47 Bandyopadhyay S, Pai SK, Gross SC, Hirota S, Hosobe S, Miura K, *et al.* The Drg-1 gene suppresses tumor metastasis in prostate cancer. *Cancer Res* 2003; **63**:1731–1736.

48 Motwani M, Sirotnak FM, She Y, Commes T, Schwartz GK. Drg1, a novel target for modulating sensitivity to CPT-11 in colon cancer cells. *Cancer Res* 2002; **62**:3950–3955.

49 Evers EE, Zondag GC, Malliri A, Price LS, ten Klooster JP, van der Kammen RA, *et al.* Rho family proteins in cell adhesion and cell migration. *Eur J Cancer* 2000; **36**:1269–1274.

50 Jaffe AB, Hall A. Rho GTPases in transformation and metastasis. *Adv Cancer Res* 2002; **84**:57–80.

51 Nauert JB, Klauck TM, Langeberg LK, Scott JD. Gravin, an autoantigen recognized by serum from myasthenia gravis patients, is a kinase scaffold protein. *Curr Biol* 1997; **7**:52–62.

52 Nicolson G. Tumor Microenvironment: paracrine and autocrine growth mechanisms and metastasis to specific sites. *Front Radiat Ther Oncol* 1994; **28**:11–24.

53 Nakajima M, Morikawa K, Fabra A, Bucana CD, Fidler IJ. Influence of organ environment on extracellular matrix degradative activity and metastasis of human colon carcinoma cells [Comment]. *J Natl Cancer Inst* 1990; **82**:1890–1898.

54 Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev* 2001; **81**:807–869.

55 Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, *et al.* Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev* 2001; **22**:153–183.

56 Morrison DK. KSR: a MAPK scaffold of the Ras pathway? *J Cell Sci* 2001; **114**:1609–1612.

57 Lozano J, Xing R, Cai Z, Jenson HL, Trempus C, Mark W, *et al.* Deficiency of kinase suppressor of Ras1 prevents oncogenic ras signaling in mice. *Cancer Res* 2003; **63**:4232–4238.

58 Yeung K, Janosch P, McFerran B, Rose DW, Mischak H, Sedivy JM, *et al.* Mechanism of suppression of the Raf/MEK/extracellular signal-regulated kinase pathway by the raf kinase inhibitor protein. *Mol Cell Biol* 2000; **20**:3079–3085.

59 Kyriakis JM, Banerjee P, Nikolakaki E, Dai T, Rubie EA, Ahmad MF, *et al.* The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* 1994; **369**:156–160.

60 Aguirre-Ghiso JA, Estrada Y, Liu D, Ossowski L. ERK(MAPK) activity as a determinant of tumor growth and dormancy; regulation by p38(SAPK). *Cancer Res* 2003; **63**:1684–1695.

61 Kennedy NJ, Sluss HK, Jones SN, Bar-Sagi D, Flavell RA, Davis RJ. Suppression of Ras-stimulated transformation by the JNK signal transduction pathway. *Genes Dev* 2003; **17**:629–637.

62 Aguirre-Ghiso JA, Liu D, Mignatti A, Kovalski K, Ossowski L. Urokinase receptor and fibronectin regulate the ERK(MAPK) to p38(MAPK) activity ratios that determine carcinoma cell proliferation or dormancy *in vivo*. *Mol Biol Cell* 2001; **12**:863–879.

63 Cole WH, Roberts S, Watne A, McGrew E. The dissemination of cancer cells. *Bull NY Acad Med* 1958; **34**:163–183.

64 Roberts SS, Watne AL, McGrath R, McGrew E, Nanos S, Cole WH. Cancer cells in the circulating blood. *Surg Forum* 1957; **8**:146–151.

65 Roberts S, Watne A, McGrath R, McGrew E, Cole WH. Technique and results of isolation of cancer cells from the circulating blood. *AMA Arch Surg* 1958; **76**:334–346.

66 Roberts SS, Watne AL, McGrath R, McGrew E, Cole WH. The response of cancer cells in the circulating blood to surgical stress and chemotherapy. *Surg Forum* 1958; **9**:595–598.

67 Oefelein MG, Kaul K, Herz B, Blum MD, Holland JM, Keeler TC, *et al.* Molecular detection of prostate epithelial cells from the surgical field and peripheral circulation during radical prostatectomy [Comment]. *J Urol* 1996; **155**:238–242.

68 Gao C-L, Rawal SK, Sun L, Ali A, Connelly RR, Banez LL, *et al.* Diagnostic potential of prostate-specific antigen expressing epithelial cells in blood of prostate cancer patients. *Clin Cancer Res* 2003; **9**:2545–2550.

69 Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, *et al.* Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P11 Study [Comment]. *J Natl Cancer Inst* 1998; **90**:1371–1388.

70 Natarajan K, Mori N, Artemov D, Bhujwalla ZM. Exposure of human breast cancer cells to the anti-inflammatory agent indomethacin alters choline phospholipid metabolites and Nm23 expression. *Neoplasia* 2002; **4**: 409–416.

71 Natarajan K, Mori N, Artemov D, Aboagye EO, Chacko VP, Bhujwalla ZM. Phospholipid profiles of invasive human breast cancer cells are altered towards a less invasive phospholipid profile by the anti-inflammatory agent indomethacin. *Adv Enz Reg* 2000; **40**:271–284.

72 Yu HG, Huang JA, Yang YN, Huang H, Luo HS, Yu JP, *et al.* The effects of acetylsalicylic acid on proliferation, apoptosis, and invasion of cyclooxygenase-2 negative colon cancer cells [Comment]. *Eur J Clin Invest* 2002; **32**:838–846.

73 Lin KH, Wang WJ, Wu YH, Cheng SY. Activation of antimetastatic Nm23-H1 gene expression by estrogen and its alpha-receptor. *Endocrinology* 2002; **143**:467–475.

74 Liu F, Qi HL, Chen HL. Effects of all-*trans* retinoic acid and epidermal growth factor on the expression of nm23-H1 in human hepatocarcinoma cells. *J Cancer Res Clin Oncol* 2000; **126**:85–90.

75 Hartsough MT, Clare SE, Mair M, Elkahloun AG, Sgroi D, Osborne CK, *et al.* Elevation of breast carcinoma Nm23-H1 metastasis suppressor gene expression and reduced motility by DNA methylation inhibition. *Cancer Res* 2001; **61**:2320–2327.

76 Yasui W, Oue N, Ono S, Mitani Y, Ito R, Nakayama H. Histone acetylation and gastrointestinal carcinogenesis. *Ann N Y Acad Sci* 2003; **983**: 220–231.

77 Ohtsuka T, Buchsbaum D, Oliver P, Makhija S, Kimberly R, Zhou T. Synergistic induction of tumor cell apoptosis by death receptor antibody and chemotherapy agent through JNK/p38 and mitochondrial death pathway. *Oncogene* 2003; **22**:2034–2044.

78 Ohtsuka T, Zhou T. Bisindolylmaleimide VIII enhances DR5-mediated apoptosis through the MKK4/JNK/p38 kinase and the mitochondrial pathways. *J Biol Chem* 2002; **277**:29294–29303.

79 Steeg PS, de la Rosa A, Flatow U, MacDonald NJ, Benedict M, Leone A. Nm23 and breast cancer metastasis. *Breast Cancer Res Treat* 1993; **25**:175–187.

80 Tagashira H, Hamazaki K, Tanaka N, Gao C, Namba M. Reduced metastatic potential and c-*myc* overexpression of colon adenocarcinoma cells (Colon 26 line) transfected with nm23-R2/rat nucleoside diphosphate kinase alpha isoform. *Int J Mol Med* 1998; **2**:65–68.

81 Miyazaki H, Fukuda M, Ishijima Y, Takagi Y, Iimura T, Negishi A, *et al.* Overexpression of nm23-H2/NDP kinase B in a human oral squamous cell carcinoma cell line results in reduced metastasis, differentiated phenotype in the metastatic site, and growth factor-independent proliferative activity in culture. *Clin Cancer Res* 1999; **5**:4301–4307.

82 Yang X, Wei LL, Tang C, Slack R, Mueller S, Lippman ME. Overexpression of KAI1 suppresses *in vitro* invasiveness and *in vivo* metastasis in breast cancer cells. *Cancer Res* 2001; **61**:5284–5288.

83 Shevde LA, Samant RS, Goldberg SF, Sikaneta T, Alessandrini A, Donahue HJ, *et al.* Suppression of human melanoma metastasis by the metastasis suppressor gene, BRMS1. *Exp Cell Res* 2002; **273**:229–239.

84 Lee JH, Welch DR. Suppression of metastasis in human breast carcinoma MDA-MB-435 cells after transfection with the metastasis suppressor gene, KiSS-1. *Cancer Res* 1997; **57**:2384–2387.