Endogenous anticancer mechanisms: metastasis

Scott Valastyan[1]

[1]Department of Cell Biology, Harvard Medical School, 200 Longwood Avenue Building C1-501, Boston, MA 02115, USA

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1. ABSTRACT

Metastases, rather than the primary tumors from which these malignant growths are spawned, are culpable for greater than 90% of human cancer-associated mortality. Metastases arise through the completion of a series of cell-biological events – collectively termed "the invasion-metastasis cascade" – which involve the dissemination of tumor cells to distant organ sites and their subsequent adaptation to these foreign microenvironments. Importantly, a number of endogenous mechanisms exist that serve to prevent metastatic progression. These safeguards must be overcome by incipient metastatic tumor cells in order for them to generate detectable metastases. Here, I highlight four endogenous mechanisms that protect against the development of metastatic disease in breast carcinomas. I discuss how the expression of these genes are dampened during malignant progression, the downstream responses they orchestrate, and clinical opportunities to therapeutically target these mechanisms. Indeed, one potentially effective strategy for the remediation of metastatic disease involves the reactivation of endogenous anti-metastasis mechanisms. Therefore, knowledge regarding endogenous anti-metastasis mechanisms may both further our comprehension of the basic etiology of metastasis and also guide the treatment of human tumors.

2. INTRODUCTION

The overwhelming majority of human cancer deaths are attributable to metastatic disease. These clinical realities are not unique to tumors originating from a single particular tissue; rather, metastasis is the root cause of patient-associated mortality in neoplasias originating from a wide spectrum of different tissues (1). It is therefore the case that our ability to effectively treat cancer is closely tied to our capacity to interdict metastatic progression. However, despite the profound clinical significance of metastasis, knowledge regarding the underlying etiology of this process remains woefully incomplete.

At a cell-biological level, metastases represent the end-products of a complex series of interrelated events often termed the "invasion-metastasis cascade", during which cancer cells in primary tumors locally invade through the surrounding basement membrane (BM), intravasate into the lumina of hematogenous vessels, survive the rigors of transport through the vasculature, arrest at anatomically distant secondary organ sites, extravasate from vessel lumina into the parenchyma of distant tissues, initially survive in these foreign microenvironments and thereby generate micrometastases, and finally re-initiate their proliferative programs at
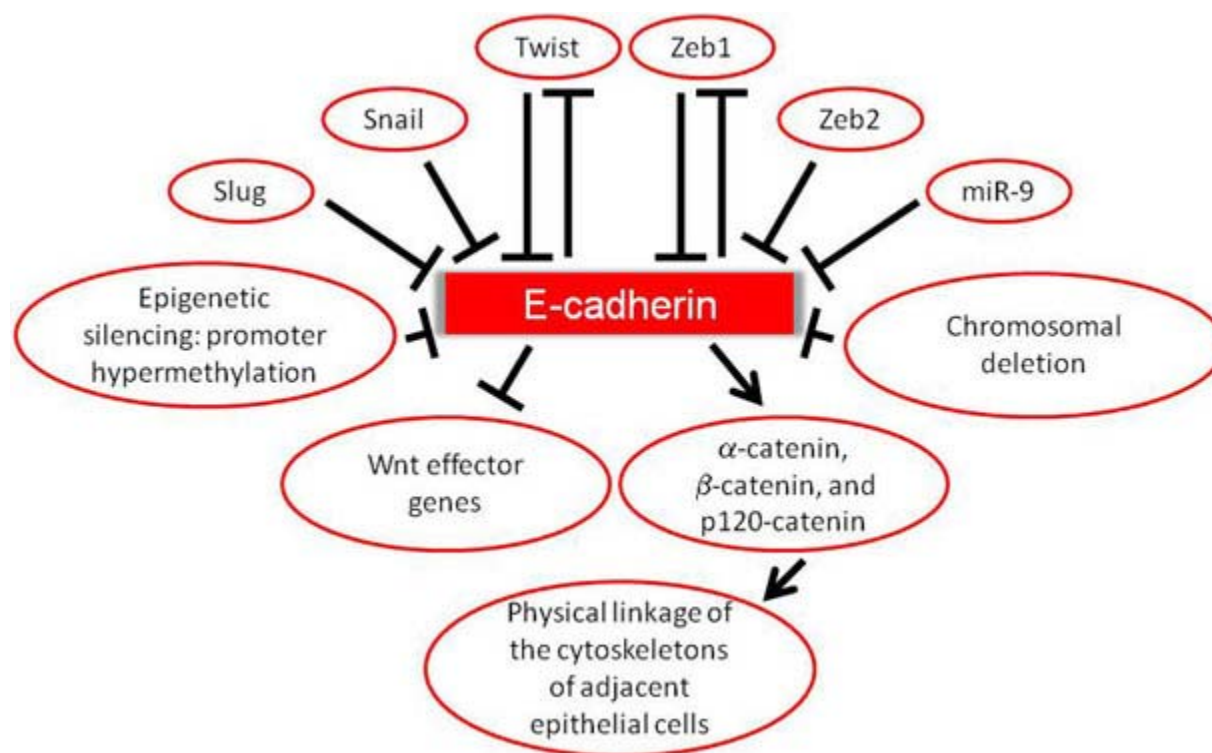
Figure 1. Signal transduction networks involved in E-cadherin-dependent endogenous anti-metastasis mechanisms. Summary of the upstream stimuli that regulate E-cadherin expression levels in malignant tumor cells, as well as the downstream effector pathways through which E-cadherin acts to control metastatic progression. miR-9: microRNA-9; Zeb1: zinc finger E-box-binding protein-1; Zeb2: zinc finger E-box-binding protein-2.

metastatic sites in order to form macroscopic and clinically detectable malignant growths (a step commonly referred to as "metastatic colonization") (2). Importantly, there exist certain endogenous mechanisms that antagonize various steps of the invasion-metastasis cascade. These mechanisms function as barriers to metastasis formation that must be overcome by incipient metastatic tumor cells in order for them to succeed in completing the invasion-metastasis cascade and, consequently, generate life-threatening macroscopic metastases.

In this Review, I highlight four well-studied examples of endogenous mechanisms that oppose metastatic progression in breast carcinomas. Increased knowledge regarding these cellular and molecular mechanisms is likely to not only enhance our basic knowledge of the invasion-metastasis cascade, but also suggest putative translational therapeutic targets that may one day prove useful for combating metastatic disease.

3. E-CADHERIN: A GATEKEEPER AGAINST THE ACQUISITION OF LOCAL INVASIVENESS

As described above, attaining an invasive phenotype is a critical early event during metastatic progression (1,2). Because carcinoma cells often invade as individual cells, one important barrier to their invasiveness is established by the epithelial origin of these neoplastic cells – more specifically, the presence of strong

intercellular junctions that link together neighboring epithelial cells into tightly integrated cell sheets (3). One prominent epithelial intercellular adhesion molecule is E-cadherin, a transmembrane glycoprotein that represents a principal component of the adherens junctions that link the actin cytoskeletons of adjacent epithelial cells (4). Thus, in order to achieve invasiveness, carcinoma cells may first be required to dissolve their existing intercellular adhesions.

As one strategy to do so, carcinoma cells are capable of co-opting an evolutionarily conserved developmental program known as the epithelial-mesenchymal transition (EMT). The EMT endows otherwise-non-invasive epithelial cells with certain fundamental properties that are characteristic of mesenchymal cells, including the dissolution of intercellular adherens junctions and the acquisition of heightened invasive capacity. Moreover, at least in certain contexts, passage through the EMT affords a means by which otherwise-non-metastatic tumor cells can attain metastatic competence. Importantly, one critical event for the EMT is downregulation of E-cadherin expression levels (4). Indeed, experimental suppression of E-cadherin suffices to induce the EMT in certain breast carcinoma cell lines (5).

In light of the imperative role played by E-cadherin during the EMT, as well as the metastasis-promoting attributes conferred by passage through the
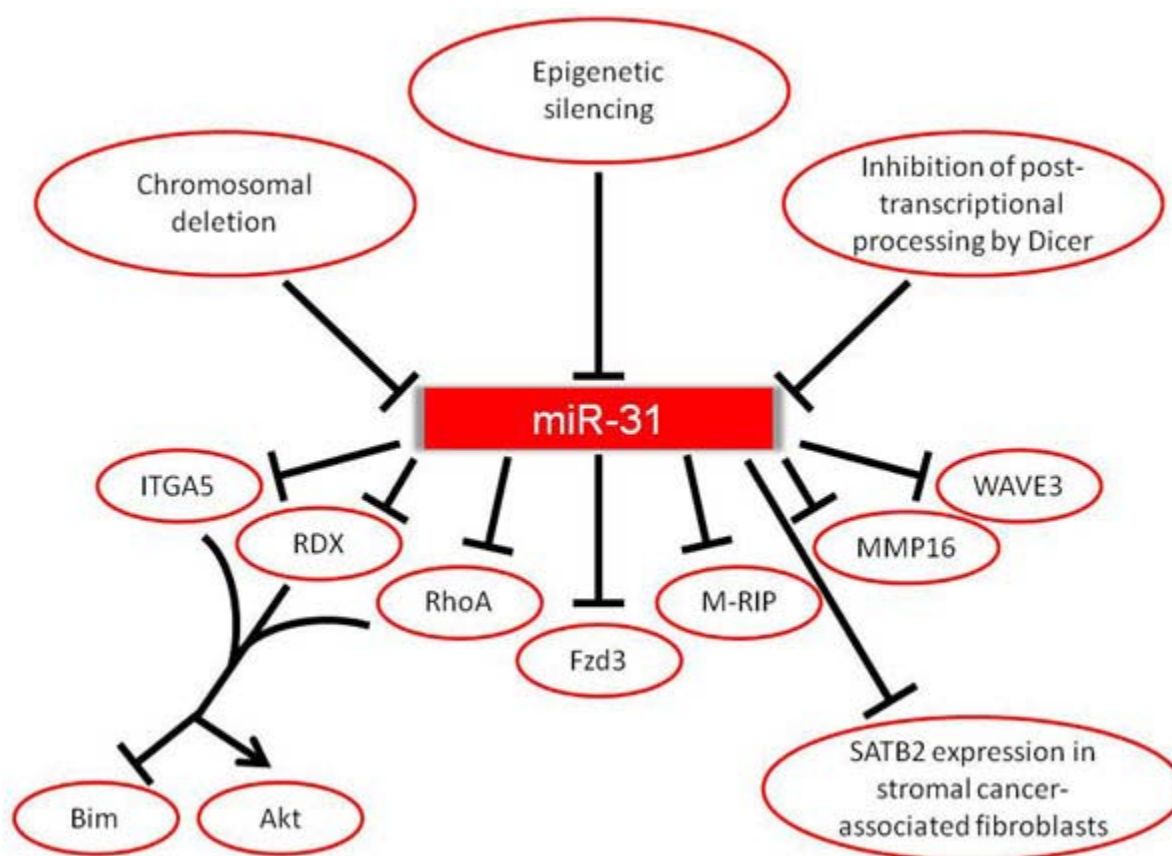
Figure 2. Signal transduction networks involved in miR-31-dependent endogenous anti-metastasis mechanisms. Summary of the upstream stimuli that regulate miR-31 expression levels in malignant tumor cells, as well as the downstream effector pathways through which miR-31 acts to control metastatic progression. Fzd3: frizzled-3; ITGA5: integrin α5; M-RIP: myosin phosphatase-Rho interacting protein; miR-31: microRNA-31; MMP16: matrix metallopeptidase-16; RDX: radixin; SATB2: special AT-rich sequence-binding protein-2; WAVE3: WASP family Verprolin-homologous protein-3.

EMT, it is perhaps not surprising that loss of E-cadherin expression can increase the metastatic potential of mammary carcinoma cells. Such differences in metastasis arise in the absence of potentially confounding influences on primary tumor development. E-cadherin's effects on metastasis have been proposed to originate due to alterations in the invasiveness of tumor cells, as well as a capacity of E-cadherin loss to affect the resistance of breast carcinoma cells to anoikis-conferred cell death upon detachment from extracellular matrix (ECM) (5,6). Interestingly, experimental suppression of E-cadherin also appears to alter metastatic colonization efficiency in breast cancer xenograft models (5). Taken together, the above-cited observations reveal that E-cadherin acts pleiotropically to impair multiple distinct steps of the invasion-metastasis cascade.

A topic of intensive research has centered upon elucidation of upstream regulatory mechanisms by which E-cadherin expression is silenced in malignant tumors. Indeed, a variety of distinct mechanisms that result in diminished E-cadherin protein levels have been reported, including transcriptional downregulation of *CDH1* (the E-cadherin-encoding mRNA), epigenetic silencing of the E-

cadherin locus via promoter hypermethylation, post-transcriptional regulation of *CDH1* by microRNAs (miRNAs), and chromosomal deletions that span the E-cadherin coding sequence (4,7,8). For example, expression of the E-cadherin-encoding mRNA is suppressed by a number of transcription factors that are known to promote the EMT, such as Slug, Snail, Twist, zinc finger E-box-binding protein-1 (Zeb1), and zinc finger E-box-binding protein-2 (Zeb2). In fact, some have proposed that the capacity of these transcription factors to stimulate entrance into a mesenchymal state is dependent on their ability to silence E-cadherin expression (4). When taken together, these discussions illustrate that carcinoma cells have devised a number of strategies by which to diminish E-cadherin levels during metastatic progression.

Another area of significant interest has involved investigating the downstream pathways through which E-cadherin achieves its biological actions during tumor evolution. The best-studied downstream networks involved in mediating E-cadherin-dependent signaling events feature the alpha-catenin-, beta-catenin-, and p120-catenin-containing adhesion complexes, which link cell-surface-localized E-cadherin to the actin cytoskeleton (4). However,
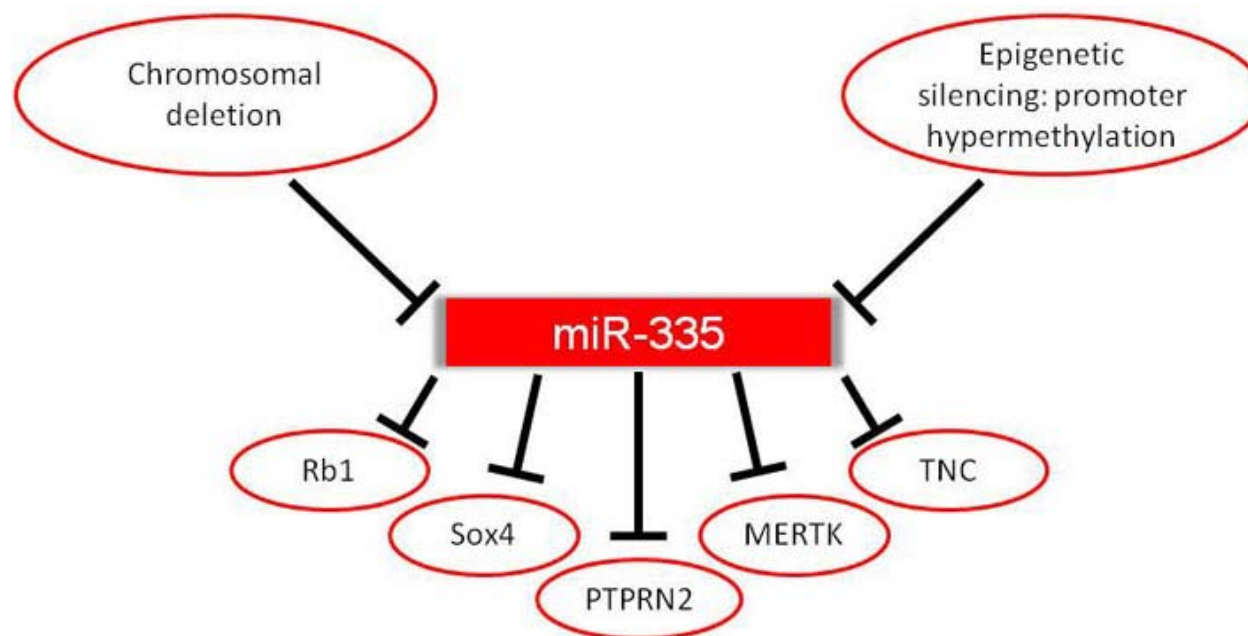
Figure 3. Signal transduction networks involved in miR-335-dependent endogenous anti-metastasis mechanisms. Summary of the upstream stimuli that regulate miR-335 expression levels in malignant tumor cells, as well as the downstream effector pathways through which miR-335 acts to control metastatic progression. MERTK: c-Mer tyrosine kinase; miR-335: microRNA-335; PTPRN2: receptor-type tyrosine protein phosphatase; Rb1: retinoblastoma protein-1; TNC: tenascin C.

E-cadherin has also been described to function in several additional, non-canonical transduction cascades. For example, experimental suppression of E-cadherin profoundly alters the global gene expression profiles of breast carcinoma cells; notably, these changes include upregulation of the EMT-promoting transcription factors Twist and Zeb1 (5). Thus, not only is E-cadherin expression downregulated by the actions of these transcription factors, but so too does E-cadherin dampen the levels of its own suppressors – thereby establishing a double-negative feedback loop. Independent of these influences, E-cadherin also impacts gene expression via its capacity to physically tether the Wnt pathway effector beta-catenin to the cell membrane – a location that is quite distant from the nucleus, where beta-catenin could otherwise localize in order to regulate the levels of various Wnt target genes. Importantly, altered activity of the above-cited E-cadherin downstream effector pathways has been linked to metastatic progression in various carcinomas (4). Collectively, these observations indicate that E-cadherin impinges upon a number of distinct metastasis-relevant signal transduction cascades.

Because of the vital role played by E-cadherin during metastatic progression, it has been proposed that therapeutic reactivation of otherwise-silenced E-cadherin may prove clinically useful. This is particularly true in the case of invasive lobular carcinomas (ILCs) of the breast, a sub-classification of breast cancer that accounts for 10%-15% of all human breast tumors and is typified by E-cadherin loss (6). Interestingly, a screen conducted to identify chemical compounds that selectively kill breast carcinomas cells with suppressed E-cadherin levels discovered that one such compound – salinomycin – effectively diminished metastasis formation in pre-clinical xenograft models. Of note, this same study found that cells with reduced E-cadherin expression were markedly less sensitive to many commonly employed chemotherapy drugs, including paclitaxel and doxorubicin (9). The above-cited studies therefore establish that E-cadherin may represent an important and viable therapeutic target in certain metastatic breast carcinomas.

4. miR-31: A PLEIOTROPICALLY ACTING SUPPRESSOR OF METASTASIS

It has recently been appreciated that – in addition to alterations in traditional protein-encoding genes – deregulation of certain non-coding RNAs can casually contribute to metastatic progression. Among these various non-coding RNAs, miRNAs have attracted the greatest amount of attention concerning their potential role in metastasis (10). miRNAs are an evolutionarily conserved family of small regulatory RNAs that suppress gene expression at a post-transcriptional level via sequence-specific interactions with the 3' untranslated regions (UTRs) of cognate mRNA targets. miRNAs are encoded within the genomes of a number of eukaryotic organisms; in fact, the human genome is estimated to contain approximately 650 different miRNA genes (11). Because an individual miRNA can concomitantly suppress the expression levels of dozens – and in some cases perhaps even hundreds – of distinct mRNA targets together in parallel, it has been suggested that greater than half of the total mRNA species encoded in the human genome are likely to be subject to miRNA-conferred regulation (12).
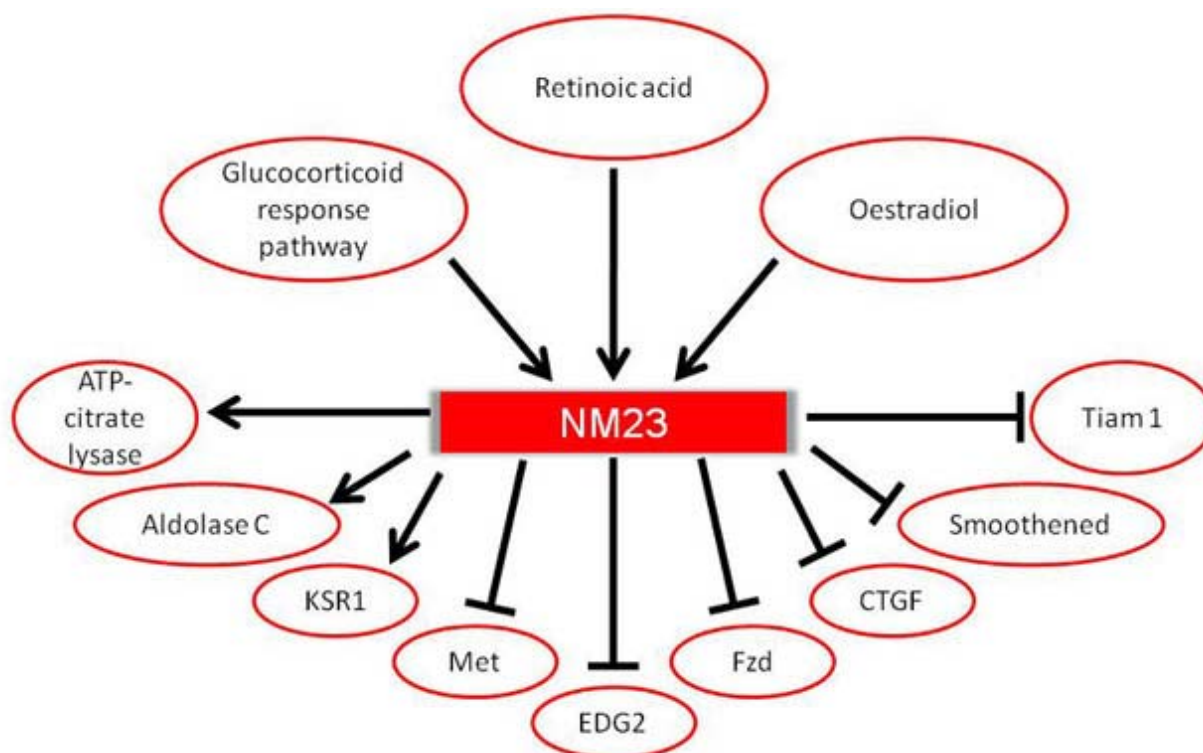
Figure 4. Signal transduction networks involved in NM23-dependent endogenous anti-metastasis mechanisms. Summary of the upstream stimuli that regulate NM23 expression levels in malignant tumor cells, as well as the downstream effector pathways through which NM23 acts to control metastatic progression. CTGF: connective-tissue growth factor; EDG2: endothelial differentiation gene-2; Fzd: frizzled; KSR1: kinase suppressor of Ras-1; NM23: non-metastatic cells protein-23; Tiam1: T-cell lymphoma invasion and metastasis-1.

Consistent with the notion that miRNAs play vital roles in the control of both physiological and pathological gene expression, global miRNA expression patterns are dramatically altered in human tumors (13). Moreover, a number of miRNAs whose perturbed expression functionally contributes to neoplastic progression have been described (10).

One such miRNA whose deregulation in tumors alters metastatic potential is miR-31. miR-31 levels are downregulated in metastatic human breast cancer cell lines and primary clinical breast tumor specimens, and the expression of this miRNA is both necessary and sufficient to inhibit metastasis in xenograft models (14,15). Notably, miR-31 exerts these anti-metastatic influences without eliciting potentially confounding effects on primary tumor formation. Of interest, miR-31 achieves its potent suppressive effects on metastasis by pleiotropically impinging upon at least three distinct steps of the invasion-metastasis cascade: local invasion, one or more early post-intravasation events (viability in the circulation, extravasation efficiency, and/or initial survival in the parenchyma of distant tissues), and metastatic colonization (15). Hence, miR-31 is capable of impeding metastasis formation via several distinct biological activities.

At present, the upstream regulatory mechanisms that lead to silencing of miR-31 expression in malignant tumors remain incompletely understood. However, one mechanism by which miR-31 levels might be downregulated in carcinomas is physical deletion of the miR-31-encoding genomic locus. Indeed, deletion of *mir-31* has been observed in a variety of types of human carcinomas (16). This observation is of particular interest in light of the fact that 9p21.3 – the chromosomal region within which the miR-31 gene resides – also harbors the genomic loci encoding several *bona fide* tumor suppressor genes (p16, p14[ARF], and p15) (17). In fact, *mir-31* is located less than 450 kb from these neighboring tumor suppressor-encoding loci (16). Consequently, even small deletions in the 9p21.3 region are likely to simultaneously abrogate the function of multiple gene products that otherwise serve to impair carcinoma pathogenesis. It is therefore striking that 9p21.3 is the single most frequently deleted chromosomal region across a wide variety of human tumors originating from a diverse array of tissue types (16,18). Moreover, it is interesting that p16 – the best-studied of the three validated 9p21.3 tumor suppressor genes – is known to be inactivated predominantly by "regional mechanisms" (e.g., chromosomal deletion or DNA methylation) rather than "local mechanisms" (e.g., point mutation) in human tumor specimens (19). I hypothesize that 9p21.3 deletions are so frequent because they represent an efficient means by

which incipient malignant cells can concomitantly abolish the functions of multiple gene products that would otherwise antagonize tumor progression.

Independent of chromosomal deletion of the miR-31-encoding genomic locus, expression of miR-31 can also be attenuated via epigenetic silencing in certain metastatic breast tumor cells (S.V. and Robert A. Weinberg, unpublished observations). Furthermore, miR-31 functional activity can be modulated at a post-transcriptional level by means of impaired processing of the miR-31 precursor RNA into its mature form. Hence, while the miR-31 precursor RNA is transcribed efficiently in a variety of cancer cell lines, overall miR-31 activity is impaired in a subset of these cells due to defective post-transcriptional processing of the miR-31 precursor RNA to its mature – and functionally active – form. Notably, this mode of regulation appears to be somewhat specific for miR-31; stated differently, while miR-31 activity is modulated by these post-transcriptional mechanisms in certain carcinoma cells, the activity of many other miRNAs in these same cells do not appear to be regulated by analogous post-transcriptional regulatory mechanisms. At least in certain cases, this post-transcriptional control appears to arise due to sequestration of the miR-31 precursor RNA in the nucleus – a location that is quite far from its site of activation-conferring endonucleolytic cleavage by the cytoplasmically confined Dicer endonuclease (20). Thus, it seems that miR-31 expression levels can be dampened via a vast array of different biochemical mechanisms during metastatic progression.

Better studied than the upstream signaling events that dictate miR-31 levels are the identity of the downstream effector pathways through which miR-31 acts to suppress metastasis. Among the many dozens of computationally predicted direct downstream targets of miR-31, a select handful appear to be particularly critical for mediating miR-31's inhibitory effects on metastasis. More specifically, it was revealed that the concomitant restored expression of three miR-31 downstream effector molecules – integrin alpha-5 (ITGA5), radixin (RDX), and RhoA – sufficed to entirely reverse miR-31-imposed metastasis suppression (21). Notably, this rescue of miR-31-dependent inhibition of metastasis reversed the known effects of this miRNA on all three steps of the invasion-metastasis cascade during which it is known to participate (local invasion, early post-intravasation events, and metastatic colonization) (21). In addition, it has been demonstrated that concurrent downregulation of the endogenous levels of ITGA5, RDX, and RhoA closely phenocopied the full spectrum of miR-31's described influences on breast cancer metastasis (22). Of interest, it appears that ITGA5, RDX, and RhoA function during at least partially unique steps of the invasion-metastasis cascade downstream of miR-31, with certain target genes (e.g., RhoA) being principally important for initial dissemination events and other effector molecules (e.g., ITGA5) functioning largely during later steps of the metastatic process (21,22).

In addition to ITGA5, RDX, and RhoA, miR-31 has also been reported to reside upstream of several other mRNAs that encode functions of apparent relevance to metastatic progression, including frizzled-3 (Fzd3), myosin phosphatase-Rho interacting protein (M-RIP), matrix metallopeptidase-16 (MMP16), and WASP family Verprolin-homologous protein-3 (WAVE3) (15,23). Intriguingly, it appears that miR-31 can act to impair the metastatic propensity of carcinoma cells via not only cell-autonomous mechanisms that operate within tumor cells themselves, but also through non-cell-autonomous mechanisms that operate within stromal cells present in the tumor microenvironment. More specifically, downregulation of miR-31 expression in cancer-associated fibroblasts (CAFs) diminished the invasive attributes of co-cultured tumor cells through a mechanism involving miR-31-imposed suppression of the homeobox gene special AT-rich sequence-binding protein-2 (SATB2) (24). When taken together, the above-cited observations indicate that miR-31 sits atop a critical metastasis-regulatory signal transduction cascade, thereby positioning this pleiotropically acting miRNA as a pivotal gatekeeper against the acquisition of metastatic proficiency.

Due to these prominent roles of miR-31 in controlling various aspects of the invasion-metastasis cascade, the suitability of miR-31 reactivation as a putative therapeutic strategy for the remediation of metastatic carcinomas has begun to be assessed in pre-clinical models. Of relevance to these discussions, clinicians have long-noted that many patients already harbor disseminated tumor cells in their bloodstream, bone marrow, and distant organs when they initially present with cancer (25,26). Therefore, truly efficacious anti-metastatic therapeutics must necessarily impair the proliferation and survival of already-established metastases, rather than merely blocking initial dissemination events. Accordingly, investigations concerning the possible therapeutic utility of miR-31-based agents have focused on the effects of acute activation of miR-31 function in already-established metastases. In breast carcinoma xenograft models, acute expression of miR-31 in already-disseminated tumor cells sufficed to both prevent the outgrowth of established micrometastases and – quite remarkably – trigger the regression of already-robustly growing macroscopic metastases. These effects of miR-31 re-introduction on the fates of already-established metastases could be accounted for by this miRNA's capacity to suppress ITGA5, RDX, and RhoA. Of interest, these signaling events seem to be transduced through Akt- and Bim-dependent biochemical pathways (27). When assessed collectively, these findings raise the possibility that miR-31-based therapeutic agents may one day prove useful for combating metastatic disease – even including, perhaps, cases involving advanced macroscopic metastases.

5. miR-335: At the Crossroads of Metastasis Suppression and Tumor-Initiating Cell Biology

It has been proposed that only a minority sub-population of the neoplastic cells present in a tumor possess the high capacity for self-renewal that is required in order for a cancer cell to seed new tumors – these hypothetically

rare cells have been termed "tumor-initiating cells" (TICs). In consonance with the TIC hypothesis are observations from certain xenograft serial transplantation studies, which have documented differential tumor-forming capacities for the various cell types that populate heterogeneous tumor masses. However, in other cases, the TIC model does not appear to be supported by empirical data thus far amassed. Nevertheless, according to the TIC hypothesis, it is ostensibly the case that one or more self-renewing TICs must disseminate during the course of metastatic progression in order for macroscopic metastases to ultimately form; in contrast, the limited self-renewal capacity of disseminated non-TICs is likely to preclude them from spawning macroscopic metastases. Indeed, certain established regulators of the process of metastatic colonization appear to be integral components of TIC-relevant self-renewal machinery (28,29).

One miRNA that suppresses breast cancer metastasis via mechanisms involving TIC biology and impaired metastatic colonization efficiency is miR-335. miR-335 levels are diminished in highly metastatic breast tumor cell sub-populations and are downregulated in human breast cancer patients that ultimately suffer disease relapse. In breast cancer xenograft assays, miR-335 expression is both necessary and sufficient to inhibit metastasis formation. Of interest, these potent effects of miR-335 on metastasis arise in the absence of potentially confounding influences on primary tumor development (30). It was originally proposed that miR-335's anti-metastatic influences were attributable to its effects on tumor cell motility; however, more recently, it has also been suggested that the anti-metastatic activities of miR-335 may be attributable to this miRNA's capacity to impair TIC-related attributes (30,31). Consequently, miR-335 can influence metastasis formation through multiple alternative cell-biological mechanisms.

The miR-335-encoding genomic locus resides at chromosome 7q32.2. Interestingly, deletion of this chromosomal region was found to be a common event in highly metastatic breast cancer cell line derivatives, as well as patient-derived breast tumor specimens. Additionally, it appears that miR-335 levels can be suppressed via epigenetic mechanisms. More specifically, hypermethylation of the DNA sequences that comprise the *miR-335* promoter region occurs frequently in highly metastatic breast cancer cells. The functional relevance of promoter hypermethylation for miR-335 silencing was demonstrated by treating highly metastatic breast cancer cells with a DNA methyltransferase inhibitor – this enhanced endogenous miR-335 levels (31). Hence, loss of miR-335 expression can be facilitated via both genetic and epigenetic mechanisms during the metastatic evolution of breast carcinoma cells.

Given the prominent role played by miR-335 during metastatic progression, the identification of downstream effectors of miR-335 that are responsible for these metastasis-related phenotypes has come to represent a topic of great interest. Notably, consistent with the proposed role of miR-335 in attenuating TIC-relevant attributes, several direct downstream effectors of miR-335 encode functions of apparent relevance to TIC biology. For example, the self-renewal-implicated transcription factor Sox4 is a miR-335 target whose suppression by this miRNA can partially account for miR-335's influences on metastatic behavior. Several other mRNAs – including those encoding receptor-type tyrosine protein phosphatase (PTPRN2), the c-Mer tyrosine kinase (MERTK), and the ECM protein tenascin C (TNC) – have also been demonstrated to represent downstream effectors of miR-335 (30).

Paradoxically, the cell-cycle progression inhibitor retinoblastoma protein-1 (Rb1) has also been reported as a miR-335 downstream target gene. These seemingly contradictory findings can be explained by the observation that miR-335-dependent suppression of Rb1 was actually growth-suppressive rather than growth-promoting, likely due to the fact that downregulation of Rb1 by miR-335 triggered activation of the p53 tumor suppressor gene and consequent cell cycle arrest (32). When taken together, the above-described data reveal that miR-335 is capable of suppressing the expression levels of several mRNAs that encode functions imperative for metastatic progression.

At present, the translational therapeutic potential of miR-335 reactivation remains unexplored. The observation that constitutive miR-335 expression impairs metastasis formation in breast cancer xenograft models is certainly encouraging; however, in light of the fact that human cancer patients frequently already harbor disseminated tumor cells at the time of initial diagnosis, further studies that assess the effects of miR-335 on already-established metastases appear warranted (25,26). Such analyses will determine whether there exists a strong impetus for pursuing miR-335 mimetics in terms of their potential capacity to antagonize metastatic outgrowth in clinically relevant settings.

6. NM23: AN ANTAGONIST OF METASTATIC COLONIZATION

From a historical perspective alone, non-metastatic cells protein-23 (NM23) is noteworthy due to the fact that it was the first identified "metastasis suppressor gene" (i.e., a gene whose encoded product inhibits metastasis without exerting potentially confounding influences on primary tumor development) (33). Also interesting are the multiple enzymatic activities possessed by NM23, which include nucleoside-diphosphate kinase activity, serine/threonine protein kinase activity, geranyl and farnesyl pyrophosphate kinase activity, histidine protein kinase activity, 3'-5' exonuclease activity, and granzymeA-activated DNase activity. These multiple biochemical functions situate NM23 well to participate in a number of cell-biological processes that impinge upon metastatic progression, such as motility and metastatic colonization efficiency. Indeed, functional studies conducted using tumor cells derived from a variety of tissues-of-origin have revealed that NM23 acts as a potent suppressor of metastasis formation in experimental model systems. Among its multiple effects

on metastasis, the capacity of NM23 to antagonize the process of metastatic colonization has attracted the greatest deal of attention (34).

Based on these observations, investigation of the molecular pathways that dictate NM23 expression levels in aggressive tumor cells have been undertaken. Detailed studies of the *NM23* promoter region have revealed that expression of the NM23-encoding mRNA is stimulated by the glucocorticoid response pathway (33,34). Indeed, NM23 expression levels were enhanced upon treatment with the glucocorticoid agonist medroxyprogesterone acetate (MPA) (34,35). In addition, NM23 expression has been reported to be positively regulated by retinoic acid- and oestradiol-dependent transduction cascades (34). Hence, NM23 expression appears to be regulated by a diverse array of signaling pathways in malignant tumor cells.

Another topic of interest related to NM23 biology has involved the identification of downstream targets of NM23 whose functions are relevant for NM23's impacts on metastatic behavior. For example, ATP-citrate lyase, aldolase C, and kinase suppressor of Ras-1 (KSR1) have been found to be substrates for the histidine protein kinase activity of NM23. Indeed, NM23-mediated suppression of KSR1 reduces signaling through the canonical extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signal transduction pathway. Additionally, microarray gene expression profiling studies have determined that the levels of several prominent signaling molecules – including the Met receptor, the lysophosphatidic acid receptor endothelial differentiation gene-2 (EDG2), the Hedgehog signaling molecule Smoothened, the Wnt pathway component Frizzled (Fzd), and connective-tissue growth factor (CTGF) – are suppressed upon NM23 expression via either direct or indirect mechanisms. Among these various downstream targets, the restored expression of only EDG2 was capable of reversing NM23-induced inhibition of cell motility *in vitro* and metastatic capacity *in vivo*. Finally, NM23 can physically interact with the Rac guanine nucleotide exchange factor (GEF) T-cell lymphoma invasion and metastasis-1 (Tiam1), thereby sequestering and inactivating it (34). Consequently, through a variety of biochemical mechanisms, NM23 is capable of perturbing downstream pathways that impinge upon several prominent aspects of metastatic progression.

As discussed above, in light of the fact that cancer patients already harbor systemically disseminated tumor cells at the time of initial diagnosis, it is increasingly appreciated that truly effective anti-metastatic therapeutic strategies must be capable of impairing the proliferation and survival of already-seeded metastases (25,26). Due to the ability of NM23 to inhibit the process of metastatic colonization, the therapeutic potential of restoring NM23 expression in already-established metastases formed by breast carcinoma xenografts was investigated in pre-clinical models. This was achieved via administration of the glucocorticoid agonist – and NM23 transcriptional activator – MPA. Quite remarkably, treatment of metastasis-bearing

mice with MPA diminished both the overall numbers and relative sizes of metastatic nodules (35). This study principally evaluated the consequences of restoring NM23 function in already-seeded micrometastases; consequently, an additional clinically important parameter for future studies involves determining the effects of MPA-induced restored expression of NM23 on the proliferation and survival of already-robustly growing macroscopic metastases. Nevertheless, on the basis of these encouraging results, phase II clinical trials centered upon utilizing MPA treatment for the remediation of metastatic disease have been initiated in patients afflicted with advanced breast carcinomas (34).

7. PERSPECTIVE

Metastases, rather than the primary tumors from which these malignant lesions arise, account for greater than 90% of human cancer deaths (1,2). Consequently, our capacity to manage the impact of cancer on human health is inextricably linked to our ability to block and/or reverse components of the invasion-metastasis cascade. Such translational objectives first require a detailed understanding of the molecular events that dictate metastatic behavior – a level of mechanistic comprehension that is only now beginning to be achieved.

Recent progress concerning genetic regulators of the invasion-metastasis cascade has revealed a number of endogenous anti-metastasis mechanisms. These mechanisms serve as important safeguards against the formation of overt metastases and, indeed, ostensibly must necessarily be overcome by incipient metastatic carcinoma cells during the course of metastatic progression. As highlighted in this Review, at least four such endogenous anti-metastasis mechanisms – involving the actions of E-cadherin, miR-31, miR-335, and NM23 – seem to function in parallel in breast carcinomas. An important direction for future work involves clarifying the extent to which these pathways similarly interdict metastasis formation in carcinomas derived from tissues other than the breast. Moreover, the identity of additional endogenous anti-metastasis mechanisms that operate in breast carcinoma cells continues to represent a topic of active research.

Given the dire clinical realities associated with the development of metastatic disease, truly efficacious anti-metastatic therapeutic agents are urgently needed. One potentially desirable therapeutic strategy involves the exploitation and reactivation of certain endogenous anti-metastasis mechanisms. Consequently, it is possible that knowledge regarding these endogenous mechanisms that serve to oppose metastatic outgrowth will not only inform our understanding of the basic etiology of metastasis, but also prove useful for the treatment of human tumors.

8. ACKNOWLEDGEMENTS

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Abbreviations: BM: basement membrane, CAF: cancer-associated fibroblast, CTGF: connective-tissue growth factor, ECM: extracellular matrix, EDG2: endothelial differentiation gene-2, EMT: epithelial-mesenchymal transition, ERK: extracellular signal-regulated kinase, Fzd: frizzled, GEF: guanine nucleotide exchange factor, ILC: invasive lobular carcinoma, ITGA5: integrin alpha-5, KSR1: kinase suppressor of Ras-1, M-RIP: myosin phosphatase-Rho interacting protein, MAPK: mitogen-activated protein kinase, MERTK: c-Mer tyrosine kinase, miRNA: microRNA, MMP16: matrix metallopeptidase-16, MPA: medroxyprogesterone acetate, NM23: non-metastatic cells protein-23, PTPRN2: receptor-type tyrosine protein phosphatase, Rb1: retinoblastoma protein-1, RDX: radixin, SATB2: special AT-rich sequence-binding protein-2, Tiam1: T-cell lymphoma invasion and metastasis-1, TIC: tumor-initiating cell, TNC: tenascin C, UTR: untranslated region, WAVE3: WASP family Verprolin-homologous protein-3, Zeb1: zinc finger E-box-binding protein-1, Zeb2: zinc finger E-box-binding protein-2.

Send correspondence to: Scott Valastyan, Harvard Medical School, Department of Cell Biology, 200 Longwood Avenue Building C1-501, Boston, MA 02115, Tel: 617-432-3971, Fax: 617-432-3969. E-mail: scott_valastyan@hms.harvard.edu.