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Linking metastasis suppression with metastamiR regulation

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Abstract

Cancer metastasis requires the coordinate expression of multiple genes during every step of the metastatic cascade. Molecules that regulate these genetic programs have the potential to impact metastasis at multiple levels. Breast cancer metastasis suppressor 1 (BRMS1) suppresses metastasis by inhibiting multiple steps in the cascade through regulation of many protein-encoding, metastasis-associated genes as well as metastasis-regulatory microRNA, termed metastamiR. In this *Feature*, we will highlight connections between BRMS1 biology and regulation of metastamiR.

Keywords

metastasis suppression; microRNA; BRMS1; metastamiR

Introduction

For cancer metastasis to develop, several processes must be completed, each requiring distinct, highly orchestrated genetic programs. Every step of metastasis is rate-limiting, meaning that when any step is blocked, a tumor cell cannot proceed to the next step. Metastasis suppressors are a group of gene products that, by definition, block metastasis but do not prevent primary tumor formation and may be targets for therapeutic intervention of the most deadly attribute of cancer cells.[1-4] The BRMS1 metastasis suppressor inhibits multiple steps of the metastatic cascade by regulating multiple metastasis-associated genes primarily through altered SIN3:histone deacetylase (HDAC) chromatin remodeling complexes.[5-8] Additionally, BRMS1 coordinately regulates multiple metastamiR (metastasis-associated microRNA), upregulating metastasis-suppressing and downregulating metastasis-promoting miRNA.[9]

microRNA (miRNA) are an expanding class of non-coding RNA genes.[10-12] miRNA regulate gene expression, predominantly at a post-transcriptional level. miRNA genes are transcribed primarily by RNA polymerase II, and form a classic hairpin motif (pri-miRNA) while being transcribed. The hairpin is recognized and cleaved by the RNAse III Drosha in conjunction

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with the co-factor DiGeorge Syndrome critical region 8 (DGCR8) to generate the pre-miRNA that is exported to the cytoplasm. The enzyme Dicer and other ribonucleoproteins further process the hairpin into a mature ~22 nucleotide miRNA that is loaded onto RNA-induced silencing complexes (RISC) to target the 3′ UTR of mRNA leading to regulation of translation.[13,14] Given the small sequence of mature miRNA, a given miR can theoretically target hundreds of mRNA, thereby making them key regulators of a multitude of normal and pathological cellular processes. Not long after their discovery in mammalian cells, miRNA dysregulation was reported in cancer. miR-15/16 were found to be frequently downregulated, or deleted, in chronic lymphocytic leukemia.[15] Since then, miRNA have been shown to play both promoting and suppressing roles in most cancers (reviewed in [ref. 16]). More recently, several miRNA have been found to regulate the process of cancer metastasis independent of primary tumorigenesis.[17]

Two recent publications prompted this *Extra View*. First, BRMS1 upregulates the metastasis suppressive miR-146a/b expression and miR-146a/b alone can suppress breast cancer metastasis.[18] Second, BRMS1 coordinately regulates expression of multiple metastamiR.[9] In this *Extra View*, we will examine the known metastamiR targets of BRMS1 and how these actions coincide with the known biology of BRMS1.

BRMS1 Regulates Metastasis-Associated Genes

BRMS1 was discovered because microcell-mediated chromosomal transfer of chromosome 11 into metastatic breast cancer cells suppressed metastasis.[19] Following this observation differentially expressed genes were identified by differential display hybridization.[20] From those analyses, full-length BRMS1 was cloned. When BRMS1 was transfected into metastatic cells, orthotopic breast, melanoma and non-small cell lung tumors were still able to grow but did not metastasize with the same efficiency.[20-24]

With regard to biological mechanism, we and others have found that BRMS1 alters several components required for different steps in metastasis: restores gap junctional intercellular communication,[25] promotes anoikis,[22] decreases both growth in soft agar[20,26] and migration/invasion.[22,23,26] At a molecular level, BRMS1 alters the transcriptome[27] and proteome[28,29] when re-expressed. These changes include, but are not limited to: epidermal growth factor receptor (EGFR;[8,30]), osteopontin (OPN;[8,31-33]), urokinase-type plasminogen activator (uPA;[34,35]), C-X-C chemokine receptor 4 (CXCR4;[36]) and fascin.[37] These changes are believed to be primarily due to the interactions of BRMS1 with SIN3:HDAC chromatin remodeling complexes.[5-8,38] Taken together, the working hypothesis for BRMS1 mechanism of metastasis suppression is as an epigenetic regulator of metastasis-associated gene expression.

MetastamiR—The Roles of miRNA go Beyond the Primary

We hypothesized that in addition to metastasis-associated protein-coding genes being regulated by BRMS1, non-coding genes, including miRNA, would also be regulated by BRMS1. Using miRNA hybridization arrays, BRMS1 was found to regulate a subset of miRNA and several of these were already described metastamiR. Interestingly, those promoting metastasis were typically decreased while those that suppressed were generally increased.[9]

Ma, Weinberg and colleagues were the first to discover and report the existence of a metastamiR, miR-10b.[39] miR-10b promoted tumor cell invasion in vitro and in vivo without affecting viability or proliferation. Importantly, metastasis was promoted in vivo (Note: Metastasis cannot be measured in vitro; only steps involved in metastasis can be modeled using in vitro assays). Other metastasis-promoting metastamiRs have since been described with functional in vivo data including: miR-21, -143, -182, -373 and 520c (reviewed in [ref. 40]).

miR-143 and -182 promoted hepatocellular carcinoma and melanoma metastasis, respectively.[41,42] miR-143 is upregulated by NFκB and decreases cellular adhesion. miR-182 stimulates migration in vitro and is part of a cluster of microRNA located on chromosome 7q31-q34 that includes miR-96 and miR-183 (miR-183-96-182). This region is frequently amplified in advanced human melanoma,[43] further supporting the role of this cluster in aggressive behavior. Interestingly, this same cluster is associated with progressive hearing loss in humans.[44,45]

An important caveat to understanding the role(s) of miR-182 in controlling cancer cell behavior is that miRNA genes are often found in close proximity on chromosomes. miRNA clusters are genetically linked and frequently transcribed from a common promoter and generate polycistronic primary transcripts.[46] As a result, individual microRNA are expressed in a coordinated network, meaning that dissecting the roles of individual microRNA within a cluster is complicated by the co-expression of the others. So, while miR-182 has been individually examined, the miR-96 and -183 may also be involved in the cancers; however, to the best of our knowledge, no one has systematically studied individual and/or combinatorial functions for any of the clustered microRNA in a functional assay. We believe that such analyses will be critical to determining the role(s) of microRNA in cancer biology.

miR-21 promoted invasion and migration, while decreasing apoptosis. Transient knockdown of miR-21 with antagomir[47] significantly decreased experimental metastasis to lungs in breast and colorectal carcinoma cells.[48] miR-373 and -520c were identified via a high throughput screen of transduced non-metastatic MCF7 human breast cancer cells with a miRNA expression library. Transductants were screened using a transwell migration assay and both miR-373 and -520c emerged as promoters of in vitro migration and invasion.[49] Experimental metastasis assays further demonstrated that these were bona fide metastamiR as both increased metastases to multiple sites.

Another class of metastamiR are those that suppress metastasis. The metastasis suppressing metastamiR include: miR-31,[50] -146a/b,[18] -206,[51] and -335.[51] Many groups have shown a role for miR-146 in inflammation.[52-55] Both miR-146a and b inhibit invasion and migration of breast cancer cells, presumably via downregulating NFκB by targeting IRAK1 and TRAF6.[53] miR-146a/b further reduce the metastatic potential of cancer cells by decreasing expression of the invasion and metastasis promoting EGFR[18] and/or signaling molecule, ROCK1.[56] These studies were extended in vivo to demonstrate that both miR-146a and b suppressed experimental metastasis in breast carcinoma cell lines.[18]

miR-206 and -335 were the first suppressing metastamiR identified. Tavazoie et al. compared miRNA expression in metastatic variants derived from the human breast carcinoma cell line, MDA-MB-231.[51] The expression of six specific miRNA were consistently low in metastatic variants. Three of them—miR-335, -126 and -206—suppressed metastasis; but, miR-126 also inhibited cell proliferation and tumorigenesis. As a result, only miR-335 and -206 can be classified as metastasis suppressors. The key step at which miR-335 and -206 suppressed metastasis was inhibition of invasion and migration.

While a majority of metastmiRs seem to play key roles in tumor cell invasion and migration, thus far only one has been shown to have roles in multiple steps of the metastatic cascade. miR-31 was recently reported to inhibit cell invasion, promote anoikis, and suppress colonization of ectopic sites,[50] leading to a 95% reduction in lung metastasis in an orthotopic model of breast cancer, while still allowing orthotopic growth. Using gene ontogeny analyses, miR-31 repressed frizzled 3 (Fzd3), integrin alpha-5 (ITGA5), myosin phosphatase-Rho-interacting protein (M-RIP), matrix metalloproteinase 16 (MMP16), radixin (RDX) and RhoA.

We acknowledge that expression of many more microRNA has been correlated with tumor aggressiveness, invasion, metastasis and survival. However, we have restricted this discussion

solely to the microRNA for which functional in vivo *data* have been published showing selective regulation of metastasis. Undoubtedly, the number of metastamiR will continue to grow.

While multiple targets have been described for each metastamiR, the end result has been either an increase or decrease in metastasis. It is interesting that a known metastasis suppressor, BRMS1, increased the expression of metastasis-suppressing metastamiR and decreases the expression of metastasis-promoting metastamiR. Still unclear is how BRMS1 is coordinately regulating so many of the genetic programs associated with metastasis. It is likely that many of the metastamiR are linked by a finite number of signaling pathways and that BRMS1 is regulating expression by both direct and indirect mechanisms.

Key Metastatic Pathways Regulated at Multiple Levels

Both metastamiR and metastasis suppressors act on selective steps of the metastatic cascade. Eleven miRNA have been shown to promote or block metastasis. This number is likely to grow because at least 20 more miRNA affect epithelial-mesenchymal transition (EMT), apoptosis and angiogenesis, all steps important in metastasis. BRMS1 represents the first metastasis suppressor to regulate miRNA. It is likewise probable that some of the 25 other metastasis suppressors will directly or indirectly influence metastamiR expression.

Especially intriguing are the similarities between BRMS1 and miR-31. Both block multiple steps of the metastatic cascade; both block colonization of ectopic tissues; and both alter expression of several metastasis-associated proteins and signaling pathways.

Those findings, coupled with an explosion of papers describing miRNA and metastasis-associated steps compelled us to expand the focus of this mini-review to consider the state of the field. Furthermore, several clinical studies have identified correlations between miRNA expression and recurrence, development of metastases and/or survival (reviewed in [ref. 57]). Therefore, our goal is to focus on the evidence for metastamirs, the implications of their existence and some technical and theoretical considerations that emerge from their discovery.

miR-10b functions to positively regulate metastasis, ultimately by activating RhoC,[39] the latter which is also decreased in BRMS1 cells.[9] The linkages between BRMS1, miR-10b and RhoC are intriguing; however, we remain cautious in our interpretation. The EMT-inducing transcription factor, TWIST1, is a positive regulator of miR-10b. BRMS1-expressing cells downregulate TWIST1 and miR-10b. Since the promoters for TWIST1 and miR-10b are still incompletely characterized, it is not yet possible to state definitively whether the BRMS1 effects are solely on TWIST1 or both TWIST1 and miR-10b.

Analogously, several of the molecules implicated in the miR-10b cascade are coincidentally regulated by BRMS1. For example, EGFR stimulates TWIST via Jak/stat signaling (but independent of Erk/MEK) to instigate the EMT.[58] BRMS1 downregulates EGF signaling both directly (by decreasing receptor expression), and indirectly (by attenuating phosphoinositide signaling).[30] Decreased EGFR expression and the TWIST-miR-10b-RhoC axis could be the result of BRMS1 regulation of miR-146a/b, both of which target EGFR.[18]

Our data, as well as the data from multiple other laboratories, clearly shows a heirearchical regulation of metastasis by BRMS1. Both direct and indirect effects on genes are operational within cells. BRMS1 can directly alter gene expression at the transcriptional level or via a transcriptional intermediate (i.e., by altering expression of a transcription factor, like TWIST). Concurrently, BRMS1 can regulate protein expression by manipulating transcription as described above, or by reducing translation by changing expression of miRNA.

Concluding Remarks

It is in a multicellular organism's best interest that cells not migrate to other sites and integrate within another tissue. Such scenarios would create chaos and disrupt homeostasis. Thus, fail safe redundancies appear to be built into the circuitry that controls cancer metastasis. However, such redundancy would come at a cost, energetically inefficient. Even though the findings emerging from our studies on cancer metastasis begin to frame an intricate regulatory network for metastasis, our findings (along with those from many other laboratories) raise several additional questions:

- Why would a cell make multiple regulators to manipulate a single molecule? What types of cross-talk exist between the various mechanisms and molecules?
- Why would a cell make a single molecule (like a miRNA) that has so many targets? What confers specificity, if there is any? What other co-factors are involved, if any? Will the stoichiometry of miRNA expression be critical?
- Would metastamiR be useful clinical targets? If so, would the promiscuity of miRNA yield more or fewer off-target effects?
- Do all of the metastasis suppressors alter metastamiR expression? Is there overlap in the metastasis suppressor ‘pathways?’
- Will exogenous miRNA have similar effects as endogenous miRNA? Are the biological consequences of re-introduction of individual miRNA artifactual when a miRNA exists within a cluster? Or should studies be done with pairs (or higher order) of miRNA?
- Are there functions of pri-miRNA and pre-miRNA in metastasis?
- What are the feedback mechanisms of miRNA (metastamiR) and metastasis suppressors and/or promoters?

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Abbreviations

RMS1

breast cancer metastasis suppressor1

HDAC
histone deacetylase

EGFR
epidermal growth factor receptor

miRNA
microRNA

miR
microRNA

pri-miRNA
primary microRNA

pre-miRNA
premature microRNA