Viewpoint

Breast cancer metastasis: a microRNA story

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Abstract

MicroRNAs (miRNAs) are small noncoding RNAs with regulatory functions, which play an important role in breast cancer. Several studies have shown that miRNAs can act either as tumor suppressors or as oncogenes, and that measurement of miRNA expression in malignancies may have diagnostic and prognostic implications. This article highlights a series of three recent studies that prove the involvement of miRNAs in breast cancer metastases. The first proves that *miR-10b* indirectly activates the pro-metastatic gene *RHOC* by suppressing *HOXD10*, thus leading to tumor invasion and metastasis. The second proves that *miR-373* and *miR-520c* can also promote tumor invasion and metastasis, at least in part by regulating the gene *CD44*. The third identifies *miR-335*, *miR-206*, and *miR-126* as suppressors of breast cancer metastasis. Loss of *miR-335* leads to the activation of *SOX4* and *TNC* (encoding tenascin C), which are responsible for the acquisition of metastatic properties. Altogether, these remarkable findings are important for our understanding of malignant transformation in the breast and may have implications for the management of patients with advanced breast cancer. The use of miRNAs as anticancer therapeutic agents is promising, and such fine molecular studies certainly help in bringing miRNAs closer to clinical practice.

miRNAs as cancer players: a balance between repression of miRNA targets and regulation of miRNA expression

MicroRNAs (miRNAs), which are short (19 to 24 nucleotides) noncoding genes that are excised from 60- to 110-nucleotide hairpin RNA precursors involved in the regulation of expression of protein-coding genes (PCGs), became a focal point in the molecular dissection of human cancer just a few few years ago [1]. They represent a new class of gene products whose functions are generally unknown. miRNA binds to target mRNA by imperfect complementarity, causing either mRNA degradation or translation inhibition. Recently, a deviation from the above perspective on miRNA function was identified; *miR-369-3* can upregulate translation of tumor necrosis factor-α [2], which suggests an additional level of complexity in miRNA function.

A growing list of reports demonstrate that miRNAs play a critical role in cancer initiation and progression, and that miRNA alterations are ubiquitous among human cancers. Consequently, events that activate or inactivate miRNAs were regarded to cooperate with PCG abnormalities in human tumorigenesis [3]. For example, it was found that miRNAs contribute to oncogenesis by functioning as downregulated tumor suppressors, as is the case for the *let-7* family, which target RAS oncogenes in lung cancers, and *miR-15a* and *miR-16-1*, which target the BCL2 oncogene in chronic lymphocytic leukemias. It was also found that miRNAs can function as over-expressed oncogenes, as is the case for the *miR17-92* cluster, which targetes the *E2F1* oncogene in lymphomas, or *miR-21*, which regulates the PTEN tumor suppressor in hepatocellular carcinomas [4]. Much less was known about the upstream regulation of miRNA in cancer cells until recently, when a series of concomitant publications showed that the TP53 tumor suppressor regulates transcription of the *miR-34* family (for review [5]) and mediate induction of apoptosis, cell cycle arrest, and senescence by TP53. Furthermore, it was shown that widespread miRNA repression by Myc contributes to tumorigenesis [6] in general and to repression of the oncogenic *miR17-92* cluster [7] in particular.

Breast cancer metastasis: a balance between suppression and activation by miRNAs

Involvement of miRNA in tumor initiation and progression has come under intense scrutiny in recent years. The involvement of miRNAs in the development of metastases was initially discovered by Ma and coworkers [8] from Robert Weinberg's group who found that *miR-10b* initiates breast cancer invasion and metastasis. Shortly afterward, Tavazzoie and colleagues [9] of the Joan Massague group revealed that *miR-335* suppresses metastasis and migration by targeting the transcription factor SOX4 and tenascin C, an extracellular matrix component. At the same time, Huang and coworkers [10] including the Reuven Agami group reported that *miR-*

miRNA = microRNA; PGC = protein-coding gene.

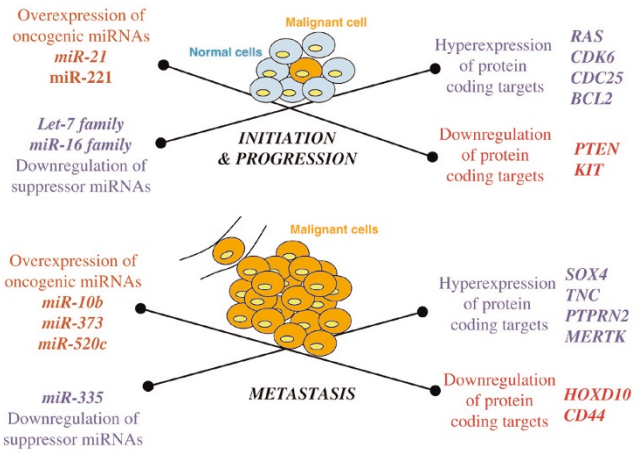
373 and *miR-520c* stimulate cancer cell migration and invasion, and proposed suppression of *CD44* (which encodes a cell surface receptor for hyaluronan) to be the underlying mechanism. Taken together, these landmark studies reveal a fine balance of noncoding RNAs as stimulators and inhibitors of metastasis, and identify several targets that could potentially represent the molecular link between miRNA deregulation and a specific tumor behavior (Figure 1).

These are remarkable discoveries, not only because they open a new field of investigation but also because the authors finely dissected molecular pathways that are involved in breast tumor metastasis (Figure 1). The study by Ma and coworkers [8] revealed that upregulation of *miR-10b* promotes invasion and metastasis. Whereas *miR-10b* is downregulated in most breast cancers in comparison with normal breast tissue [11], this miRNA is over-expressed in about 50% of metastatic breast cancers [8]. The authors proved that ectopic expression of *miR-10b* had no effect on proliferation, but an increase in transwell migration and Matrigel invasion was acquired by cells of two different human breast cell lines. *In vivo* ectopic expression of *miR-10b* conferred invasive properties on otherwise non-invasive breast cancer cells; although control tumors could not invade surrounding tissues and exhibited poor vascularization, miR-10b over-expressing tumors exhibited an invasive behavior and were highly vascularized. Moreover, although lung micrometastases were detectable in miR-10b over-expressing cells, no intravasating cells or lung metastases were found in control tumors. Together, these findings indicate that *miR-10b* can promote metastasis in otherwise nonmetastatic breast cancer cells.

Then, Ma and coworkers [8] undertook the difficult task of dissecting the molecular pathway linking *miR-10b* to the metastatic process. First, they found that Twist, a metastasis-promoting transcription factor, could induce *miR-10b* expression and demonstrated that *miR-10b* is an essential element in the Twist-induced metastasis program. Second, they proved that HOXD10, a homeobox transcription factor that promotes or maintains a differentiated phenotype in epithelial cells, is a target of *miR-10b*. HOXD10 is indeed expressed at low level in metastatic tumors. The authors demonstrated the importance of HOXD10 as an effector of *miR-10b* by showing that ectopic expression of HOXD10 that was unresponsive to miR-10b could abrogate miR-10b induced cell motility and invasiveness. Finally, the authors showed that RhoC, a G-protein involved in metastasis that is repressed by HOXD10, becomes strongly expressed in response to *miR-10b* expression. Importantly, reduction in RhoC expression by small interfering RNA caused repression of miR-10b induced cell migration and invasion, implying that RhoC is a downstream effector of *miR-10b* [8].

The work by Huang and coworkers [10] revealed that *miR-373* and *miR-520c* are also metastasis-promoting micro-

Figure 1



microRNAs and PCGs: involvement in early (initiation), intermediate (progression) and late (metastasis) steps in tumorigenesis. Presented are the specific instances in which *let-7* and *miR-16* families (already found to be involved in early stages of tumorigenesis), and *miR-21* and *miR-221* proved to be involved in tumor progression. Also shown is the newly described balance between *miR-10b*, *miR-373*, *miR-520c*, and *miR-335* in metastasis. The significant confirmed targets are also shown. miRNA, microRNA; PGC, protein-coding gene.

RNAs. *miR-373* was previously found to be associated with testicular cancer but not with metastasis. Similarly to *miR-10b*, *miR-373* and *miR-520c* did not affect cell proliferation, but promoted a migratory and invasive phenotype of MCF7 cells *in vitro*. Furthermore, MCF7 cells over-expressing *miR-373* or *miR-520c* developed metastatic nodules, which were instead absent in control cells. Elucidating the molecular pathways involved, the authors found that *miR-373* and *miR-520c* 'seed' sequences were similar, suggesting that they could regulate an overlapping set of gene targets. Among nine shared potential gene targets, *CD44* (which encodes a cell surface receptor for hyaluronan) was found to be a direct target of both *miR-373* and *miR-520c*. Most importantly, ectopic expression of an *CD44* gene that was unresponsive to *miR-373*/*miR-520c* could reduce the migratory properties of MCF7 cells over-expressing *miR-373*/*miR-520c*. To verify the significance of these results in primary breast carcinomas, the authors analyzed 11 matched normal/tumor breast samples. They found that *miR-373* was upregulated in cancer, in particular in tumors exhibiting lymph node metastasis. Moreover, an inverse correlation with CD44 expression was identified. These findings strengthen the significance of the studies performed in breast cancer cell lines.

In contrast to the other studies, the team led by Joan Massagué [9] found that *miR-335*, *miR-126*, and *miR-206* are metastasis-suppressor miRNAs. To identify these miRNAs, they compared miRNA expression of the metastatic nodules versus the unselected breast cancer parental cells. These

miRNAs were consistently downregulated in metastatic foci. Moreover, the authors found that restoring the expression of *miR-335*, *miR-126*, and *miR-106* significantly decreased the number of metastatic foci. To verify the relevance of these findings in clinical samples, Massaguè and colleagues analyzed the expression of these miRNAs in primary tumors. They found that low expression of *miR-335* or *miR-126* was significantly associated with poor metastasis-free survival. Thus, these two miRNAs were markers for the likelihood of developing metastasis. To elucidate the molecular basis of these findings, the authors profiled metastatic cells with restored *miR-335* expression. Six PGCs were suppressed by *miR-335*. Among these, knockdown of the transcription factor *SOX4* and *TNC* (encoding tenascin C) diminished *in vitro* invasive ability and *in vivo* metastatic potential, indicating that these genes are critical effectors of metastasis, which are activated by loss of *miR-335*. Importantly, in an analysis of published datasets on breast tumor gene expression, the authors found that the expression of the six signature genes was significantly associated with a poor metastasis-free survival in a cohort of 368 patients. These findings suggest the potential use of *miR-335*, its six PCG signature, and *miR-126* in prognostic stratification of breast cancer patients.

The consequences for scientists and patients: a miRevolution in breast cancer

As with all breakthrough reports, these studies - published over 4 months - have significant consequences. First, they demonstrate that combination genome-wide profiling followed by functional studies that involve over-expression and downregulation of miRNAs represent the approach that is most likely to yield advances in the field of noncoding RNA research. Abnormal expression of tumor miRNAs, characterized by differential levels of expression for mature and/or precursor miRNA sequences as compared with normal cells, has been proved to be the main abnormality, or 'miRNoma', in cancer cells. Ma and colleagues [8] found that although *miR-10b* was expressed at low levels in samples from metastasis-free patients, this miRNA was overexpressed in about half of the metastasis positive patients, a result that reconciles the data with a previous published study in which *miR-10b* was found downregulated in primary breast tumours [11]. Thus, *miR-10b* was specifically over-expressed only in metastatic cells, offering a rationale to investigate this miRNA. Furthermore, Tavazoie and colleagues [9] found that expression of *miR-335* (and *miR-126*) is lost in samples from patients with relapsed breast cancer and that this loss of expression is associated with poor distal metastases-free survival. Similarly, Huang and colleagues [10] identified significant upregulation of *miR-373* in samples from patients with metastatic breast cancer. Functional and molecular studies were essential for elucidating the significance of the abnormal expression of this miRNA in breast tumorigenesis.

A second implication of the three studies is that both the main targets and the regulators of miRNA must be identified if our understanding of the role played by miRNAs in complex multistep processes (such as primary tumor invasion and metastasis) is to improve. In the specific case of *miR-10b*, this usually difficult process was rendered possible by the extended experience of the Whitehead Institute group in unraveling genetic programs that drive tumor metastasis. Weinberg's laboratory was involved in elucidating the Twist signaling pathway during normal development and the epithelial-mesenchymal transition program, and the implications of this pathway for tumor metastasis (for review [12]). The Twist/miR-10b/HOXD10/RHOC interrelation is certainly a first example of metastasis-related interplay between noncoding RNAs and cancer PCGs. In this regard, a recent study showed that *miR-21* (a ubiquitously over-expressed miRNA in human cancers [13]) regulates cell migration and invasion in hepatocellular cancer [4]. Massague's group has a long track record of seminal discoveries related to the molecular basis of metastasis (for review [14]), whereas Agami's group has much experience in genetic screening with miRNA expression libraries, and previous elegant work by the latter [15] identified *miR-373* as an oncogene in testicular germ cells tumors through direct inhibition of the expression of the tumor suppressor LATS2. The data reported by that group clearly demonstrate that the same miRNA could target distinct PCGs in different types of tumors and work in distinct signaling pathways.

Last but not at least, this recent work could have therapeutic implications for the future. Most deaths from cancer are associated with development of metastases, and prevention or elimination of disseminated disease would have a major impact on cancer mortality. The rationale for using miRNAs as potential therapeutic targets is underpinned by the fact that miRNA over-expression in cancer cells has a pathogenic effect [16]. Therefore, future phase I trials might demonstrate reduction in *miR-10b*, *miR-373*, and *miR-520c* levels in patients with advanced breast cancer by various agents such as antisense oligonucleotides against miRNAs, looked nucleic acids, anti-miRNAs, or the antagomirs. On the other hand, over-expression of *miR-335* by mimic miRNAs could be another option to be tested in patients. Although exciting, the use of miRNA-based gene therapy in metastatic human cancers must still demonstrate high efficiency of target inhibition, with significantly improved patient survival and minimal toxicity.

Competing interests

The authors declare that they have no competing interests.

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