Reflections on miR-ing Effects in Metastasis

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In a recent issue of *Cell*, Valastyan et al. demonstrate that miR-31 can regulate multiple steps in the metastatic cascade independent of confounding effects on primary tumor development. These data have potential to provide biomarkers for prognosis and novel targets for intervention in this most lethal aspect of malignancy.

The classic view of tumor progression and metastasis is thought to involve the gradual accumulation of genetic changes and the formation of ever more aggressive tumor cells. As the field learns more about the processes of cancer initiation and progression, these ideas are changing. Clinical insights have suggested that metastasis, the often lethal spread of cells from a primary tumor to a distant site, may occur early rather than late, as previously thought (Schmidt-Kittler et al., 2003; Husemann et al., 2008; Podsypanina et al., 2008). Additionally, we now appreciate that epigenetic regulation (via alterations in microRNAs, histone modifications, and DNA methylation), as well as genetic changes, play a pivotal role in the acquisition of tumorigenic and metastatic properties (Lujambio et al., 2008; Dumont et al., 2008). Finally, the step-by-step acquisition of genetic or epigenetic changes is not necessarily needed; multiple phenotypes can be acquired with one alteration. For instance, the sole upregulation of cyclo-oxygenase 2 (COX-2) confers multiple phenotypes important in tumorigenesis such as increased proliferation, invasion, and angiogenesis as well as decreased apoptosis and immune surveillance. Likewise, in a recent issue of *Cell*, Valastyan et al. (2009) now report that the alteration of one microRNA (miRNA) modulates multiple targets and affects multiple phenotypes such as motility, invasion, and resistance to anoikis. These growing insights are very significant. With increased understanding of when and how key tumorigenic properties are acquired, the opportunity for prevention and targeted therapy increases. The metastatic cascade presents a prime example of these points.

The mechanisms that control the metastatic dissemination of tumor cells remain poorly understood. In order to successfully establish growth at a secondary site, tumor cells must first leave the primary tumor, intravasate into the systemic circulation, survive within the vasculature, extravasate into the parenchyma of distant tissues, and finally, persist long enough to allow colonization at the secondary site. As noted above, although this metastatic cascade has long been thought to be a late event in the evolution of breast cancer, there is now accumulating evidence that tumor cells can disseminate in the earliest stages of transformation. Husemann et al. (2008) have demonstrated that in the BALB-NeuT and the PyMT mammary tumor models, dissemination begins shortly after expression of the oncogenic transgene when there are no histologically detectable signs of invasion. This is also true in human disease, as disseminated tumor cells can be observed in the bone marrow of women with DCIS prior to any signs of invasive disease. Consistent with the early dissemination hypothesis, some have also reported that disseminated cancer cells in the bone marrow of breast cancer patients exhibit fewer alterations than their matched primary tumors (Schardt et al., 2005; Schmidt-Kittler et al., 2003). In addition, when untransformed mammary cells that have been engineered to express inducible oncogenes are injected into the circulation, they can develop into metastatic pulmonary lesions without the need to be transformed at their primary site of mammary glands (Podsypanina et al., 2008). Finally, the acquisition of behaviors that facilitate metastasis, such as epithelial to mesenchymal transition (EMT), can occur via epigenetic mechanisms in human cells prior to malignant transformation (Dumont et al., 2008).

Because of their ability to coordinately regulate multiple target genes and their association with clinical outcome, as first suggested by Croce and colleagues, alterations in the levels of miRNAs can play an important role in the regulation of metastasis. To gain a better understanding of metastatic dissemination, Valastyan and colleagues screened a panel of differentially regulated miRNAs based on information from the literature and now provide compelling evidence using human and murine cell lines, murine models, and assessment of clinical samples, that a specific miRNA, miR-31, acts as a metastasis suppressor and regulates multiple steps of the metastatic cascade.

This miRNA is expressed in normal breast cells, moderately reduced in non-metastatic, breast cancer cell lines, and nearly completely repressed in metastatic breast cancer cell lines of both murine and human origin as well as in breast cancer patients with metastatic disease. When examined in primary human tissues, miR-31 also demonstrated an inverse correlation with metastasis-free survival. Re-expression of miR-31 in metastatic breast cancer cells suppressed multiple surrogates of metastasis such as motility, invasion, and resistance to anoikis in vitro and suppressed metastasis following orthotopic injection into the mammary fat pads of mice in vivo. Notably, metastasis in vivo was suppressed despite the fact that miR-31-expressing breast cancer cells formed larger, more proliferative tumors. This is consistent with observations by Husemann et al. (2008), which indicated that the number and genotype of seeded tumor cells are not associated with tumor size. The authors found that the larger tumors formed by miR-31-expressing cells were well encapsulated and, thus, less locally invasive, indicating

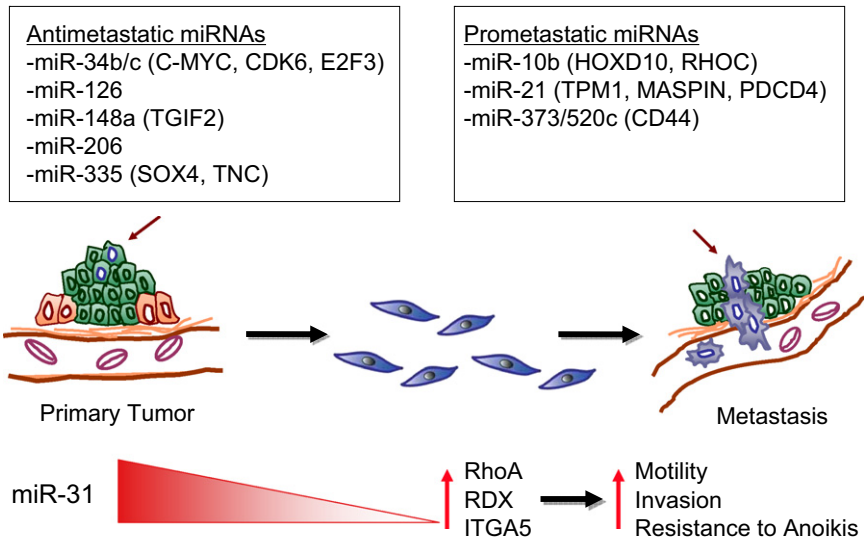

Figure 1. miR-31 Is Expressed in Normal and Nonmetastatic Breast Cells
Loss of miR-31 leads to an increase in the expression of several target genes, including RhoA, radixin (RDX), and integrin alpha 5 (ITGA5), all of which promote motility, invasion, and resistance to anoikis. Additional anti- or prometastatic miRNAs are boxed, and their known corresponding target genes are shown in parentheses. Image adapted from Kang and Massague (2004) by Drs. P. Gascard and S. Roy.

that miR-31 can exert its suppressive effects in the earliest steps of the metastatic cascade. In addition, by injecting the miR-31-expressing cells directly into the circulation to circumvent miR-31's effects on local invasion, the authors found that it also exerted its suppressive effects in later steps of the metastatic cascade, impairing the ability of the cells to survive and form secondary tumors in the lung. Thus, miR-31 exerts its antimetastatic effects at multiple steps in the metastatic cascade. These observations were also supported in converse experiments where inhibition of miR-31 via either antisense oligonucleotides or a novel miRNA sponge technique, rendered otherwise nonmetastatic breast cancer cells metastatic. Notably, inhibition of miR-31 also increased the invasive capacity and anoikis resistance of immortalized human mammary epithelial cells. This suggests that inactivation of miR-31 in normal mammary epithelium may facilitate dissemination prior to transformation to a fully neoplastic state, consistent with the aforementioned studies which now demonstrate that metastatic spread can be initiated early in the transformation process.

Collectively, the authors provide convincing data indicating that sustained miR-31 activity is necessary to prevent acquisition of aggressive traits by both tumor cells and untransformed breast epithelial cells. Importantly, unlike a number of miRNAs that have been reported to have prometastatic or antimetastatic functions (Negrini and Calin, 2008), the authors were able to demonstrate that miR-31 obstructs metastasis without confounding influences on primary tumor development and may, thus, be a true "metastasis suppressor gene," as the authors suggested.

Furthermore, by identifying a cohort of prometastatic genes regulated by miR-31, the authors also provide new insights into the mechanisms underlying metastatic spread. The coordinate repression of six of these genes, frizzled3 (Fzd3), integrin α5 (ITGA5), myosin phosphatase-Rho interacting protein (M-RIP), matrix metalloproteinase 16 (MMP16), radixin (RDX), and RhoA, correlated with more favorable clinical outcome in breast cancers and may, thus, have prognostic value. Notably, complete repression was not necessary to obtain functional effects. In addition, re-expression of three of these genes, ITGA5, RDX, or RhoA, in metastatic breast cancer cells reversed the impaired motility, invasion, and resistance to anoikis conferred by miR-31 (Figure 1). These data indicate that these genes are functionally relevant targets of miR-31. The functional relevance of RhoA was also confirmed in vivo where its re-expression was found to partially reverse miR-31-induced suppression of metastasis. This substantiates earlier findings that RhoA plays an important role in mediating EMT, which is thought to be critical in metastasis (Bhowmick et al., 2001). However, the fact that re-expression of RhoA alone could not completely reverse the defects in metastasis imposed by miR-31 highlights the involvement of multiple genes in regulating metastasis. Hence, targeting pleitropic factors such as miR-31 that can modulate several genes involved at multiple steps of the metastatic cascade would likely be a more effective therapeutic strategy than targeting a single gene. The "one hit, multiple targets" nature of miRNAs is indeed making them attractive therapeutic targets.

REFERENCES

Bhowmick, N.A., Ghiassi, M., Bakin, A., Aakre, M., Lundquist, C.A., Engel, M.E., Arteaga, C.L., and Moses, H.L. (2001). Mol. Biol. Cell *12*, 27–36.

Dumont, N., Wilson, M.B., Crawford, Y.G., Reynolds, P.A., Sigaroudinia, M., and Tlsty, T.D. (2008). Proc. Natl. Acad. Sci. USA *105*, 14867–14872.

Husemann, Y., Geigl, J.B., Schubert, F., Musiani, P., Meyer, M., Burghart, E., Forni, G., Eils, R., Fehm, T., Riethmuller, G., and Klein, C.A. (2008). Cancer Cell *13*, 58–68.

Kang, Y., and Massague, J. (2004). Cell *118*, 277–279.

Lujambio, A., Calin, G.A., Villanueva, A., Ropero, S., Sanchez-Cespedes, M., Blanco, D., Montuenga, L.M., Rossi, S., Nicoloso, M.S., Faller, W.J., et al. (2008). Proc. Natl. Acad. Sci. USA *105*, 13556–13561.

Negrini, M., and Calin, G.A. (2008). Breast Cancer Res. *10*, 203.

Podsypanina, K., Du, Y.C., Jechlinger, M., Beverly, L.J., Hambardzumyan, D., and Varmus, H. (2008). Science *321*, 1841–1844.

Schardt, J.A., Meyer, M., Hartmann, C.H., Schubert, F., Schmidt-Kittler, O., Fuhrmann, C., Polzer, B., Petronio, M., Eils, R., and Klein, C.A. (2005). Cancer Cell *8*, 227–239.

Schmidt-Kittler, O., Ragg, T., Daskalakis, A., Granzow, M., Ahr, A., Blankenstein, T.J., Kaufmann, M., Diebold, J., Arnholdt, H., Muller, P., et al. (2003). Proc. Natl. Acad. Sci. USA *100*, 7737–7742.

Valastyan, S., Reinhardt, F., Benaich, N., Calogrias, D., Szasz, A.M., Wang, Z.C., Brock, J.E., Richardson, A.L., and Weinberg, R.A. (2009). Cell *137*, 1032–1046.