

A Pleiotropically Acting MicroRNA, miR-31, Inhibits Breast Cancer Metastasis

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SUMMARY

MicroRNAs are well suited to regulate tumor metastasis because of their capacity to coordinately repress numerous target genes, thereby potentially enabling their intervention at multiple steps of the invasion-metastasis cascade. We identify a microRNA exemplifying these attributes, miR-31, whose expression correlates inversely with metastasis in human breast cancer patients. Overexpression of miR-31 in otherwise-aggressive breast tumor cells suppresses metastasis. We deploy a stable microRNA sponge strategy to inhibit miR-31 in vivo; this allows otherwise-nonaggressive breast cancer cells to metastasize. These phenotypes do not involve confounding influences on primary tumor development and are specifically attributable to miR-31-mediated inhibition of several steps of metastasis, including local invasion, extravasation or initial survival at a distant site, and metastatic colonization. Such pleiotropy is achieved via coordinate repression of a cohort of metastasis-promoting genes, including RhoA. Indeed, RhoA re-expression partially reverses miR-31-imposed metastasis suppression. These findings indicate that miR-31 uses multiple mechanisms to oppose metastasis.

For a video summary of this article, see the PaperFlick file available with the online Supplemental Data.

INTRODUCTION

Metastases account for 90% of human cancer deaths (Gupta and Massagué, 2006), yet our understanding of the molecular circuitry that governs metastatic dissemination remains fragmentary. The

invasion-metastasis cascade, which leads to these growths, is a complex, multistep process involving the escape of neoplastic cells from a primary tumor (local invasion), intravasation into the systemic circulation, survival during transit through the vasculature, extravasation into the parenchyma of distant tissues, the establishment of micrometastases, and ultimately the outgrowth of macroscopic secondary tumors (colonization) (Fidler, 2003).

MicroRNAs (miRNAs) constitute an evolutionarily conserved class of pleiotropically acting small RNAs that suppress gene expression posttranscriptionally via sequence-specific interactions with the 3' untranslated regions (UTRs) of cognate mRNA targets (Bartel, 2009). In mammalian cells, miRNAs effect gene silencing via both translational inhibition and mRNA degradation; an individual miRNA is capable of regulating dozens of distinct mRNAs, and together the >650 human miRNAs are believed to modulate more than one-third of the mRNA species encoded in the genome (Bartel, 2009).

A central role for miRNAs in the establishment and progression of human tumors has begun to emerge. More than 50% of miRNA-encoding loci reside in chromosomal regions altered during tumorigenesis (Calin et al., 2004), and expression profiling reveals characteristic miRNA signatures for many tumor types—including breast neoplasias—that predict disease status and clinical outcome (Calin and Croce, 2006). In addition, miRNAs have been identified that function as classical oncogenes or tumor suppressor genes (Ventura and Jacks, 2009), as well as a limited number that act at late stages of tumor progression (Ma et al., 2007; Tavazoie et al., 2008; Huang et al., 2008; Asangani et al., 2008; Zhu et al., 2008; Lujambio et al., 2008).

The extent to which miRNAs specifically affect metastasis remains unclear, because all the miRNAs reported to affect metastasis also exert potentially confounding influences on primary tumor development, apoptosis, and/or cell proliferation (Voorhoeve et al., 2006; Sathyan et al., 2007; Ma et al., 2007; Si et al., 2007; Tavazoie et al., 2008; Kondo et al., 2008; Lujambio et al., 2008). Moreover, a role for miRNAs in steps of the invasion-