The Metastasis Problem Gets Stickier

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The enormity and complexity of cancer genome data present significant challenges in downstream validation of novel oncogenes and tumor suppressors. In this issue of *Cancer Cell*, Hu et al. evaluate candidate oncogenes in a recurrent amplification in poor-prognosis breast cancers. They identify and validate the prometastatic gene metadherin (*MTDH*) as a key modulator of endothelial adhesion and chemoresistance.

Metastasis is a complex process involving at least four critical steps: extravasation of primary cancer cells into vasculature or lymphatics; survival of such cells in the circulation; intravasation and seeding in a new organ; and finally, growth in this new tissue microenvironment into a metastatic tumor (Gupta and Massagué, 2006). At each step, the aspiring metastatic cancer cell faces multiple obstacles, each of which is overcome with underlying epigenetic, genetic, and genomic alterations that modify the expression and function of specific metastasis-relevant genes. Based on expression profile changes, Massagué and colleagues have elegantly identified and validated four prometastasis genes—*EREG*, *MMP1*, *MMP2*, and *COX2*, which function to enhance extravasation—in both tail vein injection and orthotopic assays in immunodeficient mice (Gupta et al., 2007; Minn et al., 2005). Utilizing cross-species genomic comparisons, *NEDD9* was identified as the target of recurrent amplification in human and mouse metastatic melanomas with progression-correlated protein expression (Kim et al., 2006). Mechanistically, NEDD9 exerts proinvasive and metastatic activity in vitro and in vivo (Kim et al., 2006) and mediates the RAC1-dependent switch from amoeboid to mesenchymal cell movement, a feature of epithelial-to-mesenchymal transition (Sanz-Moreno et al., 2008). Thus, these and other examples have validated the utility of oncogenomics for the discovery of metastasis genes.

In this issue of *Cancer Cell*, Hu et al. (2009) provide evidence that the gene metadherin (*MTDH*) drives one of the steps critical for breast cancer metastasis to the lungs: adhesion to the walls of blood vessels. Using ACE (analysis of CNAs by expression data), a nearest-neighbor analysis of gene expression data, a minimal 2.9 Mb piece of chromosome 8 was found to be recurrently amplified in poor-prognosis breast cancers, a finding confirmed with fluorescence in situ hybridization (FISH) analysis. Of six genes examined in this region, only the enforced expression of *MTDH*, also known as *AEG1* (astrocyte-elevated gene 1), resulted in increased lung seeding after tail vein injection of the mildly metastatic MDA-MB-231 cell line. Conversely, knockdown of *MTDH* in the metastatic MDA-MB-231 derivative line LM2 decreased lung seeding, confirming previous studies performed in 4T1 breast cancer cells (Brown and Ruoslahti, 2004). *MTDH* overexpression had minimal effect on bone and brain metastases, suggesting tissue specificity.

Metastasis genes can impact the process via enhancing one or more steps in a highly complex cascade of biological processes. The authors eliminated a role for MTDH in intravasation or extravasation using both in vitro and in vivo assays. Instead, *MTDH* overexpression was shown to enhance breast cancer cell adhesion to lung endothelial cells in an in vitro attachment assay, while knockdown produced the opposite effect in three independent breast cancer cell lines. Conceptually, this enhanced adhesion could lead to increased deposition of cancer cells in the lung, providing a statistically higher likelihood that deposited cells will bloom into overt metastases (Figure 1). Indeed, a previous study showed a higher colocalization of MTDH-positive metastatic cells with lung endothelial cells (Brown and Ruoslahti, 2004).

Hu et al. also demonstrate an effect of MTDH on chemoresistance. Overexpression decreased and knockdown sensitized breast cancer cells to cell killing by paclitaxel, doxorubicin, cisplatin, and hydrogen peroxide in vitro. Again, multiple cell lines showed this effect, demonstrating the general function of MTDH in chemoresistance. These results were extended to an in vivo subcutaneous tumor assay for both paclitaxel and doxorubicin. By using overexpression and knockdown assays, the effect on chemoresistance was shown to be partially mediated by *ALDH3A1* and *MET*, two genes whose expression levels were significantly altered by modulation of MTDH. Thus, in a clinical context, cells with increased MTDH levels could be positively selected for during a course of chemotherapy. This microevolutionary stress, along with selection for other factors, could iteratively refine the primary and/or circulating tumor cells for full metastatic propensity (Figure 1). Importantly, Hu et al. connected MTDH to human patient data, showing that MTDH amplification/overexpression correlates with breast cancer patient survival independently of several classical risk factors such as ER negativity or HER2/neu positivity.

The Hu et al. study contains many of the requisite steps required of a rigorous candidate gene validation effort, namely: (1) the use of multiple cell lines to demonstrate functional activities, ensuring that the observed consequences are not artifacts of a particular cell line; (2) mutually reinforcing and complementary functional data, i.e., reciprocal overexpression and knockdown data; (3) use of multiple model systems, including in vitro and in vivo assays; (4) examination of DNA, RNA, and

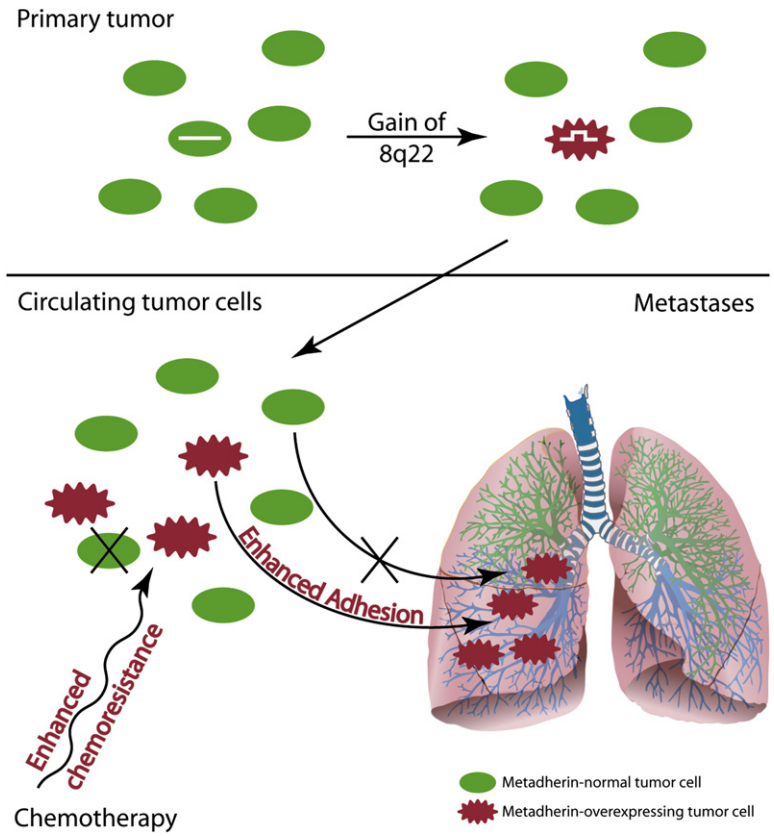

Figure 1. Dual Role of *MTDH* in Breast Cancer Chemoresistance and Endothelial Adhesion
Amplification of 8q22 or overexpression of metadherin (*MTDH*) by other means results in a breast cancer cell that is both more resistant to chemotherapy and more adhesive to blood vessels lining the lung. This one-two punch could explain a higher rate of metastasis in patients with *MTDH*-overexpressing cells. The lung illustration is in the public domain and was retrieved from http://commons.wikimedia.org/wiki/Image:Respiratory_system_complete_en.svg.

protein to cover the major levels of gene regulation; (5) exploration of putative downstream effectors; and (6) establishment of relevance to human tumor specimens. Such a multipronged, stringent approach is necessary for establishing the biological relevance of a novel cancer gene if such studies are to advance the field.

The Hu et al. study also highlights a key challenge in cancer genomics. The proverbial haystack of candidate genes has grown exponentially in the era of large-scale cancer genomics. The search for needles—those novel cancer genes that will lead to new therapeutics and diagnostics for cancer patients—has thus become ever more daunting. Rigorous validation and mechanistic exploration of a single genetic element of interest (GEOI), as in this study and elaborated elsewhere (Chin and Gray, 2008), is time and labor intensive, representing a major bottleneck. Rapid translation of a novel target or molecular diagnostic to the clinic therefore demands that the cancer research community be equipped and ready to prioritize and focus its resources on the highest potential candidates. Here, we need to employ high-throughput biological assays or functional genomic screens that can systematically evaluate many GEOIs in a single experiment so that the cancer relevance of a GEOI can be assigned based on not only the genomic and genetic but also the biological weight of evidence.

In designing functional genomic screens, one should be mindful that cancer is a multigenic disease that evolves in a host microenvironment and that no single model is capable of fully recapitulating the complex biology of cancer in a human patient. For example, the "experimental metastasis" assay involving forcibly injecting cancer cells into the tail vein to look for lung colonization is commonly used as a proxy of metastasis; however, in reality this captures only one late aspect of this complex cascade. Similarly, in vitro invasion assays in modified Boyden chambers assess only the ability of a cancer cell to leave the primary tumor site and invade into the surrounding microenvironment, not its ability to enter the circulation or to grow in a foreign soil. Mutually reinforcing data from both models, against the backdrop of genomic and correlative data in human cancers, will increase our confidence in the biological relevance of any given GEOI. In other words, we should recognize that each model has pros and cons, and that such limitations necessitate the complementary uses of disparate models. Thus, we must invest in the development and characterization of comprehensive experimental model systems that can be used to simulate the diverse genetic and cellular contexts in which cancer evolves to enable informative functional genomic screens.

In summary, the studies of Hu et al. provide another strong testament to the power of a comprehensive atlas of the genomic architecture of cancer to pinpoint novel genes critical to the development and progression of lethal cancers. At the same time, they underscore the need for high-throughput functional genomic screens and reinforce the importance of multiple in vitro and in vivo model systems for translating insights from large-scale cancer genomics.

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