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Stepping on the GAS: a brake pedal for melanoma metastasis?

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Metastatic melanoma is notoriously refractory to most medical therapies; therefore, elaborating the biological basis of metastasis in melanoma may speed the development of novel therapeutic approaches that reduce mortality from this malignancy. Although recent years have witnessed considerable advances in our understanding of its molecular underpinnings, factors that specifically modulate the metastatic process in melanoma remain incompletely understood. To acquire metastatic potential, cancer cells must learn to invade into their surrounding matrix, survive passage through the bloodstream or lymphatics, and successfully colonize secondary sites. In doing so, they enact various metastasis-promoting genes while down-modulating other genes, termed metastasis suppressor genes, that inhibit this process (reviewed by Rinker-Schaeffer et al., 2006). However, few scalable experimental systems have been developed to query the key regulatory components of this multi-step phenomenon *in vitro*.

The advent of RNAi-based methodologies for directed gene silencing in mammalian systems, together with the availability of genome-wide “short hairpin” RNAi (shRNA) libraries have provided unprecedented avenues for systematic interrogation of cancer-associated phenotypes. In recent years, the stable introduction and screening of pooled shRNA libraries using retroviral or lentiviral vectors has yielded numerous new biological insights relevant to several tumor types. By employing microarray-based “half hairpin” DNA barcodes to identify shRNAs that have become enriched or depleted following selections, pooled RNAi screens offer an increasingly accessible means to interrogate tumor processes in an unbiased fashion and at a genome scale (Schlabach et al., 2008).

Recently, Gobeil et al. (2008) employed a genome-wide shRNA screening approach to identify genes that modulate metastasis in melanoma. The investigators introduced a series of retroviral shRNA pools (collectively spanning >68,000 shRNAs and ~28,000 genes) into B16-F0 mouse melanoma cells, which are weakly metastatic, followed by implantation of these cells into a three-dimensional collagen/matrigel plug. In this assay, highly metastatic cell lines form satellite colonies around the primary plug, whereas poorly metastatic lines do not. When cells containing the shRNA pools were implanted, enhanced satellite colony formation was observed compared to inactive shRNA controls. The investigators isolated these colonies and sequenced the shRNAs therein to identify putative metastasis suppressor genes in melanoma.

Of 78 candidate genes that emerged from this primary screen, 22 were confirmed to suppress the metastasis of B16-F0 cells, based on enumeration of lung metastases following tail vein injections of B16-F0 derivatives in which the relevant genes were silenced by individual shRNAs. Among those genes, only *GAS1* (Growth arrest-specific 1) exhibited downregulated gene expression in the B16-F10 cell line, a highly metastatic derivative of the B16-F0 cells used in the primary screen. Subsequent experiments demonstrated that

GAS1 manifests key properties characteristic of metastasis suppressor genes: its knockdown increased metastasis formation in a spontaneous metastasis assay, and its expression promoted apoptosis upon dissemination to secondary sites of growth. Finally, decreased *GAS1* expression was observed in metastatic melanoma samples from a gene expression profiling study, and in a histochemical analysis of a lymph node metastasis relative to its primary melanoma counterpart.

The identification of *GAS1* as a possible melanoma metastasis suppressor gene is intriguing on several levels. *GAS1* is a cell surface protein that facilitates the binding of Sonic Hedgehog (SHH) to PTC1, thereby activating the SHH pathway. Since increased SHH signaling augments tumor growth in several contexts, *GAS1* expression might have been expected to promote metastasis. This apparent paradox may be explained in part by observations that the growth regulatory function of *GAS1* may operate independently of its role in SHH signaling. In line with this notion, *GAS1* expression has been demonstrated to inhibit growth of some human cancer cell lines. Moreover, ectopic *GAS1* did not affect SHH signaling in the melanoma model system employed by Gobeil and colleagues. Thus, these observations may open a new line of study into regulation of cell growth control in the tumor metastasis setting.

The findings of Gobeil et al. also raise interesting questions regarding the broader relevance of *GAS1* as a metastasis suppressor in human melanoma. On a genomic level, the *GAS1* locus (chromosome 9q21.3-q22 in the human genome) is commonly deleted in metastatic melanomas (Lin et al., 2008), as might be expected for a metastasis suppressor gene. On the other hand, it could be argued that at least some of this genetic loss is attributable to the presence of the *CDKN2A* locus on chromosome 9p. *CDKN2A* deletion is one of the most common genetic aberrations in melanoma, and many such deletion events actually involve the entirety of chromosome 9. Furthermore, chromosome 9 deletions are equally common in primary melanomas, suggesting that genetic events involving this locus are not specific to the metastatic phenotype. Epigenetic studies of the *GAS1* promoter in primary and metastatic melanomas, together with additional expression-based studies in larger melanoma tumor collections, should help refine the specificity of *GAS1* silencing in metastatic melanoma.

Beyond the role of *GAS1* in melanoma metastasis suppression, the study by Gobeil and coworkers demonstrates the growing importance of systematic shRNA screens for functional interrogation of increasingly complex cancer phenotypes. At the same time, the successes of genome-scale RNAi highlight several technological challenges inherent to this methodology. In the Gobeil study, for example, less than one third of the candidate genes (or “hits”) from the primary shRNA screen could be confirmed through validation *in vivo*. Here, “hit” identification was based on a single shRNA per gene, which brings to mind the well-recognized “off-target” shRNA effects that increase false positive rates in many RNAi screens. This problem can be mitigated in part by mandating that multiple shRNAs targeting the same gene “score” in a primary screen. Conversely, the knockdown efficacy of shRNAs from most screening libraries is often incomplete, which may also elevate false negative rates in these screens. These limitations notwithstanding, genome-scale pooled shRNA screening holds tremendous promise for the provision of biological insight into numerous mechanisms that control tumorigenesis and metastasis. The recent foray into the biology of metastasis suppression by Gobeil et al. provides the latest demonstration of the discovery power of high-throughput RNAi when applied to a robust and biologically informative *in vitro* model system.

References

- Lin WM, Baker AC, Beroukhi R, Winckler W, Feng W, Marmion JM, Laine E, Greulich H, Tseng H, Gates C, Hodi FS, Dranoff G, Sellers WR, Thomas RK, Meyerson M, Golub TR, Dummer R, Herlyn M, Getz G, Garraway LA. Modeling genomic diversity and tumor dependency in malignant melanoma. *Cancer Res.* 2008; 68:664–673. [PubMed: 18245465]
- Rinker-Schaeffer CW, O'Keefe JP, Welch DR, Theodorescu D. Metastasis suppressor proteins: discovery, molecular mechanisms, and clinical application. *Clin Cancer Res.* 2006; 12:3882–3889. [PubMed: 16818682]
- Schlabach MR, Luo J, Solimini NL, Hu G, Xu Q, Li MZ, Zhao Z, Smogorzewska A, Sowa ME, Ang XL, Westbrook TF, Liang AC, Chang K, Hackett JA, Harper JW, Hannon GJ, Elledge SJ. Cancer proliferation gene discovery through functional genomics. *Science.* 2008; 319:620–624. [PubMed: 18239126]