From Integrated Genomics to Tumor Lineage Dependency

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Abstract

In principle, genomic information derived from tumors should illuminate critical cellular dependencies that are tractable to therapeutic targeting; however, realizing this ideal remains difficult. Using an integrated analysis of high-resolution single nucleotide polymorphism maps and gene expression databases associated with the NCI60 collection cancer cell lines, we identified the transcription factor MITF as an amplified oncogene in melanoma that is critical for anchoring lineage dependence and malignant character. Similar combined genomic approaches may be useful in other cancer types to learn how critical regulators of tumor lineage are linked to genomic alterations in cancer cells. (Cancer Res 2006; 66(5): 2506-8)

Introduction

The clinical promise of molecular cancer therapeutics rests on the knowledge of the genetic lesions and cellular mechanisms that are critical for maintaining malignant character. Thus, it is important to identify and classify "sensitive" tumor subgroups by genetic criteria to maximize the utility of the small but growing number of drugs that can target various molecular pathways (1–3). This recognition has prompted widespread application of genome-technologies, such as high-throughput sequencing and DNA microarrays, which can allow a comprehensive interrogation of cancer genomes at an unprecedented scale and resolution.

Despite these advances, several challenges confront large-scale efforts to identify salient and "target-able" tumor mechanisms. Cancer genomes frequently contain dozens of genetic changes that may involve hundreds of genes, many of which represent bystander events unrelated to carcinogenesis or tumor progression. Therefore, discerning causal tumor mechanisms represents a significant obstacle to translational cancer genomics. Moreover, informative global genomic surveys should ideally account for the increasing evidence that biologically pertinent perturbations affect a restricted set of hallmark processes that direct cell growth and survival (4). If the panoply of genetic aberrations observed in human cancer converges onto a much smaller set of critical "tumor dependencies," then genome-scale methods to fully elucidate these dependencies are needed to enhance the development and application of rational molecular therapeutics.

With this ideal in mind, the challenge of cancer genomics is to define critical tumor dependencies by deconvoluting the information provided by complex genomic data sets. Efforts to achieve this end will be markedly enhanced by computational and experimental approaches that streamline hypothesis generation and enable robust validation within appropriate cellular contexts. Accordingly, the integration of multiple, matched genome-scale data sets may yield more refined hypotheses than does analysis of chromosomal or gene expression data alone. To be rigorous, functional genomics studies should also employ model systems that not only reflect relevant tumor biology but also provide an experimentally accessible framework to verify the tumor dependencies predicted by in silico analyses. Linking genomic signatures and tumor dependencies in this manner will allow the application of molecular therapeutics in a more rational and effective manner.

To begin to develop integrative methods along the lines described above, we initially used the NCI60 panel of cancer cell lines (5). The NCI60 collection represents a unique model system for this purpose because multiple large-scale NCI60 data sets, including gene expression data and pharmacologic profiles, are already available (6, 7). To enhance these existing data sets, we first derived high-resolution genomic maps (including chromosomal gains, losses, and loss-of-heterozygosity) for 58 NCI60 cell lines using high-density single nucleotide polymorphism microarrays (8). Hierarchical clustering of single nucleotide polymorphism array copy number data suggested that some NCI60 cell lines, including six of eight melanoma lines, might be characterized by lineage-restricted patterns of copy number alterations. To examine whether such genetic events might harbor lineage-specific cancer genes, we performed a combined analysis of NCI60 copy number and gene expression data using supervised learning methods. These efforts led to the discovery that the microphthalmia-associated transcription factor (MITF) gene is amplified in a subset of melanomas (9). In primary tumors, MITF amplification was associated with metastatic disease and with decreased patient survival. In vitro functional studies showed that MITF cooperated with the mutated protein kinase B-raf (BRAF) to transform immortalized human melanocytes, thereby confirming its ability to function as an oncogene in a relevant cellular context. Moreover, antagonism of MITF activity using a dominant negative adenoviral construct both suppressed melanoma cell growth in vitro and sensitized these cells to cytotoxic agents. MITF therefore seemed to drive a critical tumor survival mechanism that operated in a subset of melanomas.

Although it was already known as a master regulator of the melanocyte lineage (10), our study was the first to define an oncogenic role for MITF in melanoma. However, there is clear precedent for tumor-promoting properties within the basic helix-loop-helix/leucine-zipper superfamily and MiT subfamily of transcription factors to which MITF belongs. Prominent examples include MYC (11) and its homologue NMyc, the latter of which undergoes frequent applications in pediatric neuroblastomas and medulloblastomas (12, 13). Additionally, the MiT transcription factors encoded by TFE3 and TFEB are both targeted by chromosomal translocations observed in papillary renal cancer and soft-tissue sarcomas, resulting in gene fusions with PRCC or other partners (14–16). Thus, the oncogenic role of MITF is shared with MiT transcription factors in certain cell lineages.

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Nonetheless, the role we defined for MITF in survival of melanoma cells was unexpected because previous studies showed that its forced overexpression induces cell cycle arrest in primary melanocytes (17, 18). Thus, melanoma cells that elaborate this oncogenic mechanism must presumably contain additional alterations that uncouple the prosurvival and growth-inhibitory effects of MITF. In support of this expectation, Loercher et al. (17) have observed that silencing of the cyclin-dependent kinase (CDK)N2A (p16) locus enables melanocytes to escape MITF-induced growth arrest. Additionally, all NCI60 cell lines that displayed copy gain at the MITF locus exhibited inactivating mutations in the p16/CDK4/Rb pathway (19). Lastly, the cooperative transforming effect of MITF and BRAF(V600E) was shown in immortalized melanocytes that expressed the INK-insensitive CDK4(R24C) variant, which phenocopies p16/CDK4/Rb pathway disruption (9). These observations highlight a melanoma tumor dependency mechanism that may involve aberrant MAP kinase activation, inactivation of the Rb pathway, and deregulated expression of MITF (Fig. 1). This dependency not only integrates key genetic lesions in melanoma but also highlights a set of intriguing therapeutic targets, including mitogen-activated protein (MAP) kinase and its effectors, cyclin-dependent kinases, and MITF target genes, such as BCL-2 and HIF-1α (refs. 20, 21; Fig. 1).

Overall, the discovery of MITF function in melanoma reveals a newly recognized “lineage survival” or “lineage addiction” subtype of oncogene that may prove to be relevant in many kinds of cancer. Lineage addiction bears some resemblance to “oncogene addiction,” which describes a gain-of-function mutation required for both carcinogenesis and tumor maintenance (22). Rather than enacting gain-of-function mechanisms specific to the tumor, lineage survival oncogenes may instead target master survival functions required for the relevant cellular lineage during development and differentiation. A retrospective view of the cancer literature readily identifies other known oncogenes that may also mediate essential lineage survival functions. For example, the well-characterized oncoprotein cyclin D1 promotes cell cycle progression in tumors while also enabling maturation of the mammary cell lineage (23). Similarly, the FLT3 tyrosine kinase represents both an important tumor survival factor in acute leukemia and a critical player in myeloid differentiation (24). Moreover, the androgen receptor provides critical transcriptional signals guiding both normal prostate epithelial differentiation and prostate cancer survival/progression (25). Each of these lineage dependencies offers therapeutic targets with proven efficacy (androgen receptor and FLT3) or substantial promise (CCND1/MITF effector proteins; Fig. 1). In summary, genome-scale and functional analyses that can integrate the

Figure 1. Combined genomic analysis identifies a melanoma lineage survival mechanism. A major goal of cancer genomics research is to classify human tumors according to biologically and therapeutically informative molecular criteria. We did an integrated analysis of sample-matched, genome-scale data sets derived from NCI60 cancer cell lines (top). This approach identified MITF as an amplified melanoma oncogene. Subsequent experiments showed that MITF may cooperate with BRAF(V600E) to transform melanocytes (see text). Together with aberrant MAP kinase activation and p16/Rb pathway inactivation, MITF may direct a lineage dependency mechanism operant in a subset of melanomas (bottom) and vulnerable to therapeutic interdiction at several points (red). pRb, retinoblastoma protein.
information found in complex genomic data sets should permit the identification of tumor dependency mechanisms that are currently invisible to simpler analyses. Our use of the NCI60 cell line database also offered one perspective on chemical genetics studies of chemotherapeutic sensitivity that proved to be useful. The above observations suggest that the combination of multiple, sample-matched genomic data sets together with genome-wide functional studies may speed the discovery of clinically identifiable, biologically informative, and therapeutically tractable dependencies in many forms of cancer.

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References
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