"Lineage Addiction" in Human Cancer: Lessons from Integrated Genomics

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Genome-era advances in the field of oncology endorse the notion that many tumors may prove vulnerable to targeted therapeutic avenues once their salient molecular alterations are elucidated. Accomplishing this requires both detailed genomic characterization and the ability to identify in situ the critical dependencies operant within individual tumors. To this end, DNA microarray platforms such as high-density single-nucleotide polymorphism (SNP) arrays enable large-scale cancer genome characterization, including copy number and loss-of-heterozygosity analyses at high resolution. Clustering analyses of SNP array data from a large collection of tumor samples and cell lines suggest that certain copy number alterations correlate strongly with the tissue of origin. Such lineage-restricted alterations may harbor novel cancer genes directing genesis or progression of tumors from distinct tissue types. We have explored this notion through combined analysis of genome-scale data sets from the NCI60 cancer cell line collection. Here, several melanoma cell lines clustered on the basis of increased dosage at a region of chromosome 3p containing the master melanocyte regulator *MITF*. Combined analysis of gene expression data and additional functional studies established *MITF* as an amplified oncogene in melanoma. MITF may therefore represent a nodal point within a critical lineage survival pathway operant in a subset of melanomas. These findings suggest that, like oncogene addiction, "lineage addiction" may represent a fundamental tumor survival mechanism with important therapeutic implications.

Cancer results from a diseased genome. Each tumor contains a collection of genomic aberrations that activate oncogenes and inactivate tumor suppressor genes. A recent survey of the scientific literature identified 229 oncogenes (or "dominant" cancer genes) and 62 tumor suppressors ("recessive" cancer genes), suggesting that more than 1% of the human genome may contribute directly to carcinogenesis and/or tumor progression (Futreal 2004). Since many tumor mechanisms likely remain undiscovered, these numbers may underestimate the full spectrum of human cancer genes. Moreover, the path to cancer may require at least 5–10 genetic mutations (Hahn and Weinberg 2002). Theoretically, then, the total number of different genetic combinations possible across all human cancers exceeds ten trillion and may even reach 10^{18}. These estimates imply that a comprehensive genomic approach to cancer therapeutics may be exceedingly difficult to achieve.

Recent insights, however, suggest a more favorable conclusion: The enormous complexity possible in theory may indeed prove both functionally reducible and therapeutically tractable in practice. Among these is the recognition that most human cancers derive from perturbations within a finite number of fundamental physiological processes directing cellular proliferation, survival, angiogenesis, and invasion/metastasis (Hanahan and Weinberg 2000). By itself, this conceptual framework does not completely resolve the challenge of tumor complexity, because many diverse genetic players and mutation chronologies may affect each of these properties. Nonetheless, the notion that cancer involves definable biological hallmarks suggests that, ultimately, logic and order may be discerned from the immense genomic diversity characteristic of human cancer once the appropriate molecular contexts are more fully understood.

Consistent with this viewpoint is the recognition that cancer genomic aberrations, although complex, do not occur randomly. Instead, a relatively small number of cancer genes tend to undergo alterations at high frequencies. The fact that cellular pathways involving RAS, p53, and pRb (among others) undergo genetic mutations so commonly (Vogelstein and Kinzler 2004) not only endorses the "hallmarks of cancer" model, but also suggests that cancers tend to employ the same genomic alterations to enact these processes. Thus, despite the inevitable complexity, an increased knowledge of cancer genomic alterations should contribute markedly to the elaboration of essential and broadly applicable tumor mechanisms.

ONCOGENE ADDICTION AND TUMOR DEPENDENCY

Another pivotal insight pertaining to deconvolution of cancer genomic complexity derives from the recent observation that some tumors require continued activity of a single activated oncogene for survival (Weinstein 2002). Termed "oncogene addiction," this phenomenon was first demonstrated in transgenic mouse models that enabled conditional overexpression of oncogenes such as *myc*, *ras*, and *bcr-abl* (Chin et al. 1999; Felsher and Bishop

1999; Huettner et al. 2000; Jain et al. 2002; Pelengaris et al. 2002). In these models, induction of the relevant oncogene triggered cancer formation; however, subsequent loss of oncogene expression resulted in regression and apoptosis of tumor cells. The presence of oncogene addiction in human malignancies was first demonstrated in chronic myelogenous leukemia (CML), which harbors the BCR-ABL translocation; and in gastrointestinal stromal tumors (GIST), which contain oncogenic mutations in the c-kit gene. Targeting the tyrosine kinase activity of these oncogenes with the small-molecule inhibitor imatinib was sufficient to induce complete remissions in the great majority of patients (Druker et al. 2001; Demetri et al. 2002; Kantarjian et al. 2002). More recently, oncogene addiction was also demonstrated in a subset of lung cancers that contain base mutations or small deletions in the epidermal growth factor receptor (EGFR) gene; these alterations confer sensitivity to EGFR inhibitors such as gefitinib or erlotinib (Lynch et al. 2004; Paez et al. 2004). Thus, a single oncogenic lesion may play a decisive role in tumor maintenance, even when many additional genetic alterations have also accrued (Kaelin 2004).

A synthesis of the oncogene addiction and "hallmarks of cancer" models offers a framework wherein massive apparent genetic complexity may be underpinned by a much smaller collection of critical "dependencies" operant in human tumors. By this view, the predicted tumor-promoting effects of many genomic perturbations may converge onto a finite number of physiological processes, which in turn exhibit an even smaller set of limiting "nodes" or "bottlenecks" within key cellular pathways directing carcinogenesis. At the same time, these dependencies will likely be caused by or associated with identifiable genetic lesions. Thus, such concordant genomic events may allow tumor dependencies to be pinpointed in situ within individual tumor samples (Fig. 1). Such an approach would markedly enhance efforts toward targeted therapeutic interdiction; however, most critical tumor dependencies remain either undiscovered or invisible to current molecular pathological tools.

GENOMIC APPROACHES TO CANCER CHARACTERIZATION

The tumor dependency framework and the genomic basis of cancer suggest that in the future, targeted cancer therapeutic avenues will depend primarily on rigorous genetic definition (Weber 2002). Recent years have therefore witnessed numerous efforts toward comprehensive characterization of tumor genomic alterations. The most popular large-scale approaches to cancer characterization utilize DNA microarrays to profile the expressed genes within tumor samples (Ramaswamy and Golub 2002). Accordingly, gene expression studies of many tumor types have identified molecular subclasses based on unique mRNA signatures, suggesting that the goal of a complete "molecular taxonomy" of human cancer is achievable (Golub 2004).

In principle, elaborating this molecular taxonomy should also clarify salient tumor dependencies and

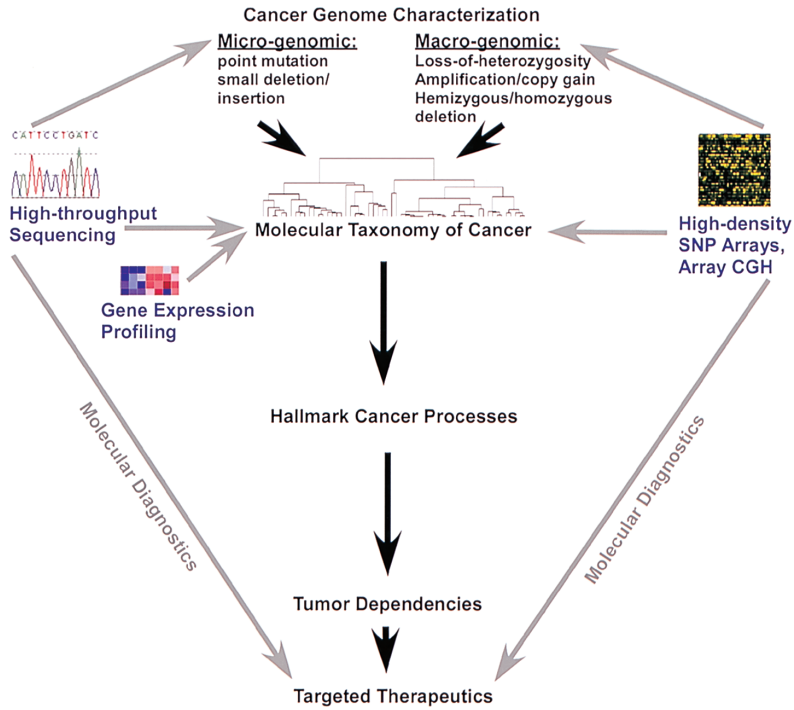

Figure 1. Cancer genomics and enabling technologies. The goal of high-resolution cancer genome mapping is the comprehensive identification of molecularly defined tumor types. Optimally, these subtypes will also clarify hallmark biological process and tumor dependencies that guide future targeted therapeutic avenues. High-throughput sequencing and microarray-based tools for RNA and DNA analysis (e.g., gene expression profiling, SNP arrays, and array CGH) represent important technologies for cancer genomic analyses.

thereby identify targetable cellular pathways directing tumor survival (Fig. 1). DNA microarrays have proved tremendously successful in identifying mRNA signatures predictive of a wide range of biological and clinical phenotypes. However, the explanatory power of these signatures with regard to the underlying biology remains variable. Instead, factors such as the degree of stromal/inflammatory infiltration within tumor samples and lineage-specific transcriptional programs may heavily influence the composition of microarray-derived signatures. In particular, cell lineage effects on gene expression often dominate the output of unsupervised analytical techniques such as hierarchical clustering or self-organizing maps (Ross et al. 2000; Ramaswamy et al. 2001) unless these studies are confined to samples from a single lineage.

A complementary approach to molecular cancer classification involves the systematic global analysis of tumor DNA. This avenue offers conceptual appeal given the genomic origins of human tumors. The structural alterations characteristic of cancer genomes are more refractory to technical variables (tissue hypoxia, media conditions, etc.) and agnostic to lineage- or differentiation-dependent transcriptional programs. Moreover, prevalent tumor DNA alterations tend to harbor oncogenes or tumor suppressors, and therefore, in combination with expression profiling, may speed the identification of critical tumor growth or survival mechanisms on a genome scale.

Enabling technologies such as high-throughput sequencing and DNA microarrays have propelled recent efforts at DNA-based cancer genomic analysis (Weber 2002). Large-scale sequencing provides detailed characterization of "micro-genomic" alterations (e.g., base mutations or small deletions/insertion events) (Weir et al. 2004), whereas microarray-based tools such as oligonucleotide comparative genomic hybridization (CGH) have facilitated studies of "macro-genomic" alterations (translocations, copy gains or losses spanning many kilobases) (Mantripragada et al. 2004; Garraway and Sellers 2005).

Recently, high-density DNA microarrays that perform massively parallel genotyping of single-nucleotide polymorphisms have become available (Cutler et al. 2001). Although these SNP arrays (Affymetrix) were designed for large-scale association studies in medical or population genetics, they have proved robust and versatile tools for cancer genome analysis. SNP arrays contain oligonucleotide probes tiled to detect the two alleles of a given SNP locus. The current generation SNP array contains probe densities capable of genotyping >100,000 SNPs simultaneously, providing a median intermarker distance of 8.5 kb. This high array marker density enables the inference of tumor loss-of-heterozygosity (LOH) events, even in the absence of matched normal samples (R. Beroukhim et al., in prep.). Moreover, analysis of the signal intensities that result from genomic DNA hybridization, and comparison to corresponding signal data from normal genomes, allow determination of copy number changes present within tumor samples at high resolution, as shown in Figure 2 (Bignell et al. 2004; Zhao et al. 2004).

LINEAGE-RESTRICTED DNA ALTERATIONS IN HUMAN TUMORS

To explore the utility of global cancer genome analysis using high-density SNP arrays, we applied an unsupervised learning algorithm to DNA copy number information derived from SNP array studies (Garraway et al. 2005). Here, we used 100K array data from the NCI60 cancer cell line collection as a model system (Stinson et al. 1992). This panel includes 59 cell lines from nine different tumor types accrued by the National Cancer Institute (NCI). NCI-sponsored studies of these lines have also generated pharmacological data for nearly 100,000 compounds (data from >40,000 compounds are publicly available through the NCI Web site at http://dtp.nci.nih.gov). Additional large-scale data sets available for this collection—including several gene expression microarray surveys (Ross et al. 2000), spectral karyotyping (SKY)

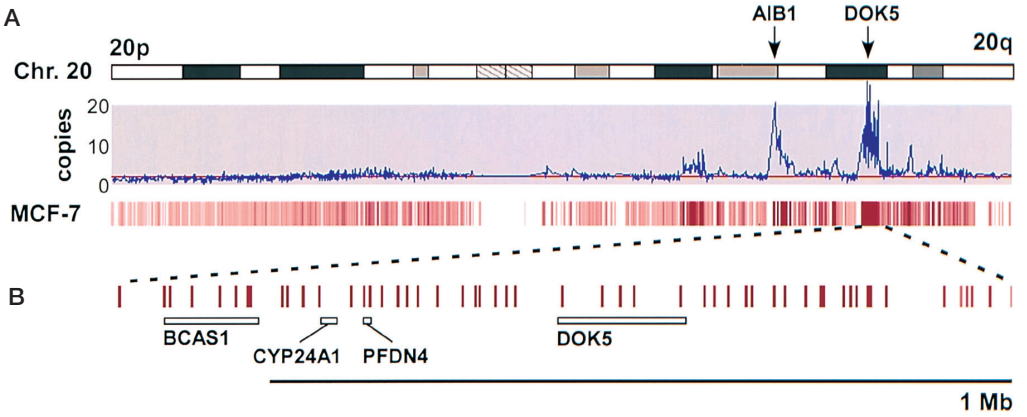

Figure 2. High-density SNP arrays for copy number analysis. (*A*) A SNP array-derived copy number plot (*middle*) of the MCF-7 breast cancer cell line is shown alongside the cytoband map of chromosome 20 (*top*). Marker density and signal intensities are indicated by the white-red colorgram (*bottom*). AIB1 and DOK5 are genes present within two high-level amplicons. (*B*) Expanded view of a 20q amplicon shows the 100K array marker density and a map of several genes located therein (*bottom*). (Reprinted, with permission, from Garraway and Sellers 2005 [©Elsevier].)

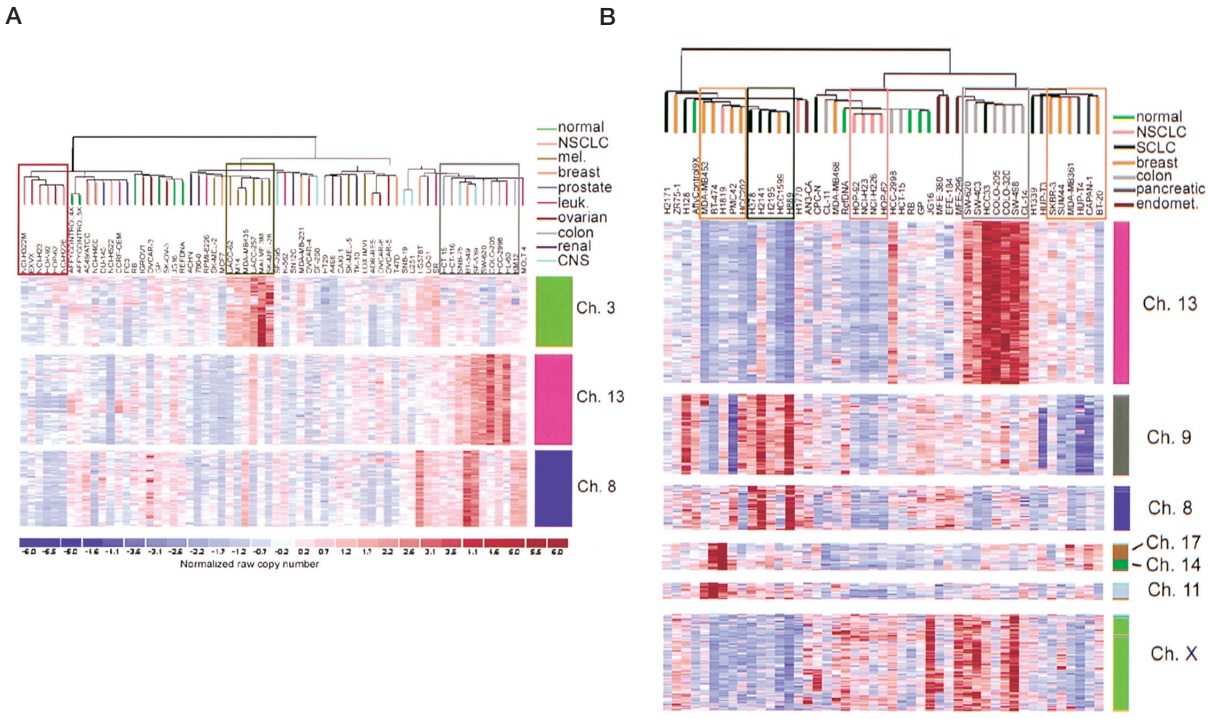

Figure 3. Copy number clustering of cancer cell lines. (*A*) Hierarchical clustering was applied to SNP array-based copy number data from 64 NCI60 cell lines and controls. (*B*) Dendrogram and associated SNP probe clusters from an independent cancer cell line set (several NCI60 cell lines were also included for confirmation). For both *A* and *B*, the resulting dendrograms (*top*), along with SNP probe clusters from correlated genomic regions (*bottom*), are shown. Columns represent cell lines, and each row (pixel) represents an individual SNP marker. Terminal branches are color-coded according to tissue type (*legend at right*). Pixel color represents copy number data (*red* = increased copy number and *blue* = decreased copy number). Lineage-enriched cell line subclusters are outlined by colored rectangles. (*A*, Reprinted, with permission, from Garroway et al. 2005 [©Nature Publishing Group].)

(Roschke et al. 2003), and proteomic profiles (Nishizuka et al. 2003)—make the NCI60 panel an attractive system in which to integrate large, orthogonal data sets and to query their respective biological importance.

When hierarchical clustering (Eisen et al. 1998) was used to group the NCI60 collection based on copy number alterations, the resulting cell line dendrogram contained three major branches, each consisting of samples from several different tumor types (Fig. 3A) (Garraway et al. 2005). Surprisingly, closer inspection of these branches revealed terminal subclusters where the cell lines appeared to segregate according to tissue of origin (e.g., non-small-cell lung cancer, melanoma, and colon cancer lines; Fig. 3A). These results suggested that despite the marked ploidy variances known to exist within these cell lines, their genomes might nonetheless harbor lineage-associated aberrations discernible by the unsupervised learning algorithms in common use.

To confirm this observation while also excluding a spurious phenomenon attributable to experimental batch effects, we carried out an independent SNP array hybridization that included a subset of NCI60 samples alongside 35 non-NCI60 cancer cell line DNAs within a single experimental batch. The resulting copy number information was also subjected to hierarchical clustering as described above. Lineage-restricted cell line subclusters were again observed, in this case consisting of breast, colon, small-cell, or non-small-cell lung cancer lines (Fig. 3B). Together, these data raised the possibility that tissue lineage may exert a significant effect on patterns of copy number alterations in cells from many different tumor types.

To investigate the in vivo relevance of the lineage-restricted patterns observed in cell lines, we also examined the copy number patterns of a large collection of lung tumor DNAs analyzed by 100K SNP arrays (Zhao et al. 2005). Since these samples were processed and hybridized to arrays in several batches, the raw copy number values at each SNP locus were first converted to integer values by a hidden Markov model. Next, a prevalence threshold for amplifications and deletions was calculated by taking the mean frequencies plus one standard deviation of all SNPs having inferred copy number ≥ 4 and ≥ 1, respectively. SNP loci exceeding either threshold were filtered using dChipSNP software and subjected to hierarchical clustering. These manipulations effectively removed the "batch effects" that may confound clustering analysis of "raw" copy number data, while enriching for the genomic regions most likely to denote tumor subtypes.

The lung cancer samples organized into three discernible aggregates following hierarchical clustering of the regions described above, as shown in Figure 4. Interestingly, the lung cancer cell lines formed a cluster that was distinct from those of the tumor samples. This finding resembled prior observations from gene expression studies (Ross et al. 2000) and suggested that distinctive

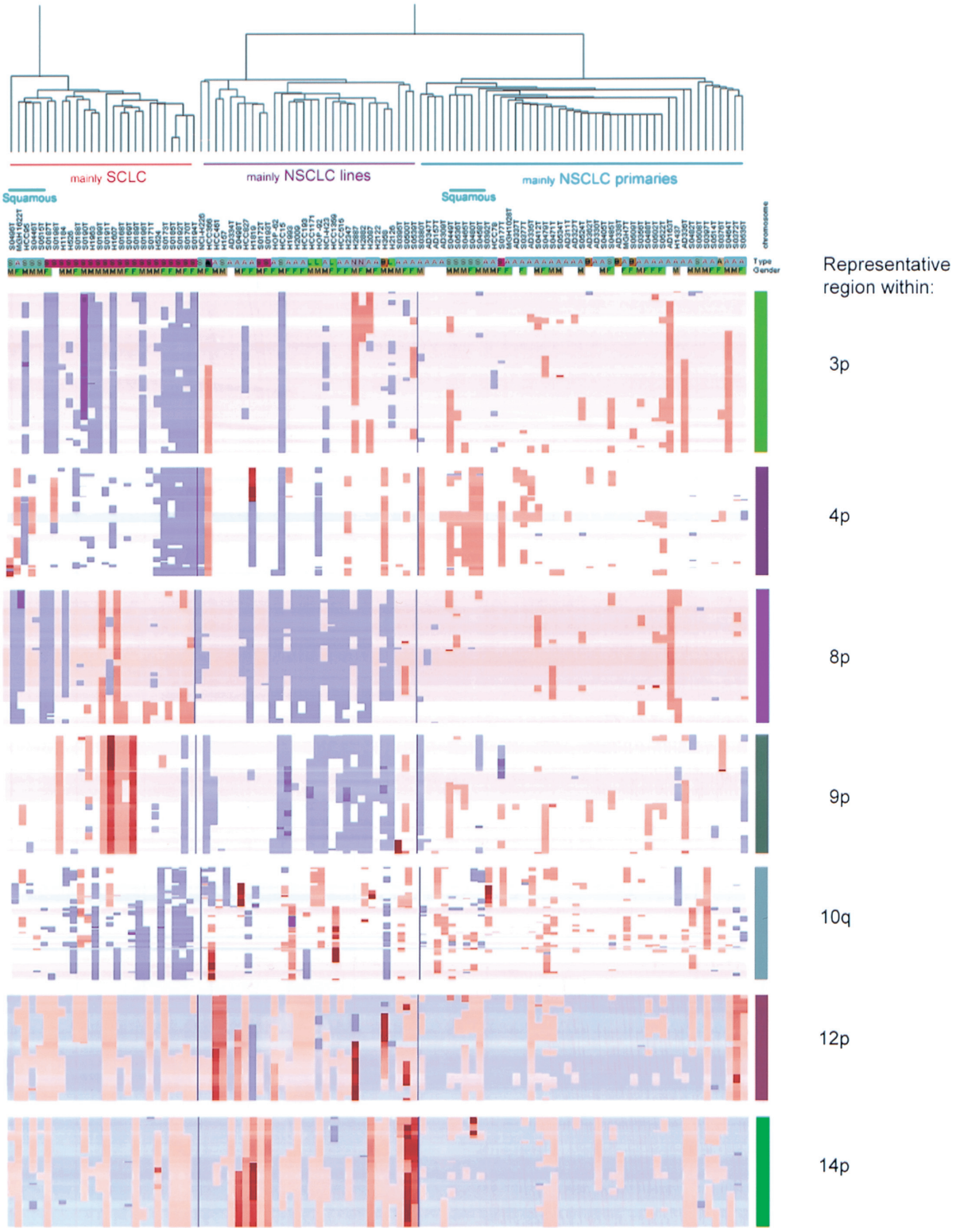

Figure 4. Copy number clustering of human lung tumors. A prevalence threshold for both amplification and deletion was calculated; SNP loci exceeding either threshold were filtered and used for inferred copy number clustering. The resulting dendrogram and selected SNP clusters from correlated chromosomal regions are illustrated. White areas represent mean copy number (copy number values for each SNP were standardized to have a mean of 0 and standard deviation of 1), blue areas represent copy number below the mean, and red areas represent copy number above the mean.

copy number alterations may either accompany or result from established in vitro cultivation. Nonetheless, lineage-restricted patterns were again apparent: The small-cell lung cancer (SCLC) samples segregated mostly within a single cluster, whereas the non-small-cell lung cancers (NSCLC; mostly adenocarcinomas) formed a

separate cluster. Although the squamous cell carcinoma samples did not form their own branch, they did comprise two terminal subclusters discernible within the larger SCLC or NSCLC clusters (Fig. 4). In general, these findings accord well with a recent meta-analysis of comparative genomic hybridization studies performed across many tumor types (Greshock et al. 2005) and suggest that cell or tissue lineage may exert a profound influence on the patterns of chromosomal aberrations observed within human tumors.

INTEGRATED GENOMIC ANALYSES IDENTIFY A LINEAGE-DEPENDENT MELANOMA ONCOGENE

Hierarchical, copy-number-based clustering algorithms applied to cancer cell lines were two-dimensional; as such, they also grouped SNPs deriving from the same chromosomal regions (Figs. 3,4). Inspection of these SNP clustering patterns identified specific genomic loci whose copy number alterations appeared to drive the terminal cell line subclusters. For example, the colon cancer cluster was largely based on copy number gain within chromosome 13 in both experiments (Fig. 3A,B), whereas the melanoma group reflected copy gain within chromosome 3 (Fig. 3A; *note:* no melanoma cell lines were included in the sample batch depicted in Fig. 3B).

The lung tumor clustering analysis also identified candidate genomic regions. For example, distinct SCLC subsets were associated with losses at chromosomes 3p, 4p, and 10q, consistent with prior studies (Fig. 4) (Levin 1995; Petersen 1998; Sattler and Salgia 2003). In contrast, NSCLC cell lines more commonly exhibited losses at chromosomes 8p and 9p (Fig. 4). Interestingly, additional tumor subsets appeared to cluster on the basis of alterations not previously described in lung cancer, such as a region of gain at chromosome 4p associated with a squamous carcinoma subset (Fig. 4). Presumably, genomic regions correlated with the lineage-restricted copy number clusters may harbor cancer genes directing key mechanisms governing tumor growth in these subtypes.

Cancer cell lines exhibiting lineage-dependent copy number alterations provide experimentally tractable model systems for the characterization of associated oncogenes or tumor suppressor genes. However, in most cases the aberrations identified by hierarchical clustering were too large for detailed functional studies. Even the smallest minimal common regions so identified were several megabases in length, and some cases exhibited low-level (e.g., single-copy) gain or loss involving much of a chromosome arm (polysomy).

We therefore applied an integrated genomic approach to identify candidate cancer genes located in these regions (Garraway et al. 2005). Here, we reasoned that the oncogene target of a genetic amplification event present in a set of samples might exhibit significantly increased steady-state gene expression when compared to samples lacking this amplicon. This rationale derived from several predicted properties of amplified oncogenes: (1) preferential (over)expression in tumor cells; (2) enrichment by clonal selection relative to bystander genes; and (3) deregulation, e.g., increased refractoriness to negative feedback/regulatory mechanisms that might otherwise suppress a gene dosage effect. Since gene expression data from several groups are publicly available for the NCI60 cell lines, as noted above, this sample collection provided a convenient platform for an integrated approach.

To combine gene expression and copy number information in this way, we adapted supervised learning methods commonly utilized for microarray-based tumor classification (Golub et al. 1999). NCI60 samples were separated into two classes based on the presence or absence of a genomic lesion linked to lineage-restricted subsets identified by copy number clustering. Next, NCI60 gene expression data (generated on the Affymetrix U95 platform by the Genomics Institute of the Novartis Foundation) were organized according to these class distinctions to identify genes with significantly increased expression in association with the amplified class. Finally, highly expressed genes within the "amplicon" (or copy gain) class were mapped to their genomic locations to determine whether any also resided within the amplified segments.

As noted above, one of the NCI60 subsets identified by copy number clustering consisted exclusively of melanoma cell lines (Fig. 3A). This cluster correlated best with DNA copy gain at chromosome 3p14-3p13. When we applied the integrated genomic approach to the class distinction 3p-amplified versus non-amplified, only one gene was both significantly up-regulated in association with the amplified class (following Bonferroni correction) and located within the common region of copy gain that defined this class (Fig. 5A). This gene, *MITF*, belongs to the MiT family of helix-loop-helix/leucine-zipper transcription factors and effects critical functions in the development and survival of the melanocyte lineage (Goding 2000; Widlund and Fisher 2003). Although *MITF* itself was not previously shown to be altered in cancer, it had been implicated as a transcriptional regulator of the antiapoptotic *BCL2* oncogene in melanoma cells (McGill et al. 2002). Moreover, other bHLH-LZ and MiT transcription factors undergo genetic alterations causally implicated in several human malignancies; these include *MYC*, the prototype amplified oncogene; *NMYC*, amplified in 50% of pediatric neuroblastomas and associated with adverse outcome (Maris and Matthay 1999); and both *TFE3*, and TFEB, the targets of translocation-mediated gene fusions with *PRCC* or other partners in papillary renal cancer and soft-tissue sarcomas (Weterman et al. 1996; Ladanyi et al. 2001; Davis et al. 2003). In light of these observations, our genomic analysis suggested that *MITF* might function as a lineage-specific melanoma oncogene.

Several lines of experimental evidence have since confirmed the oncogenic function of MITF in human melanoma (Garraway et al. 2005). Quantitative genomic PCR performed on a series of DNAs derived from primary and metastatic melanomas demonstrated *MITF* copy gain (to ≥4 copies) in 10% of primary and more than 20% of metastatic specimens. Analysis of a melanoma tissue array by fluorescence in situ hybridization (FISH) revealed similar findings (Fig. 5B) and also enabled a

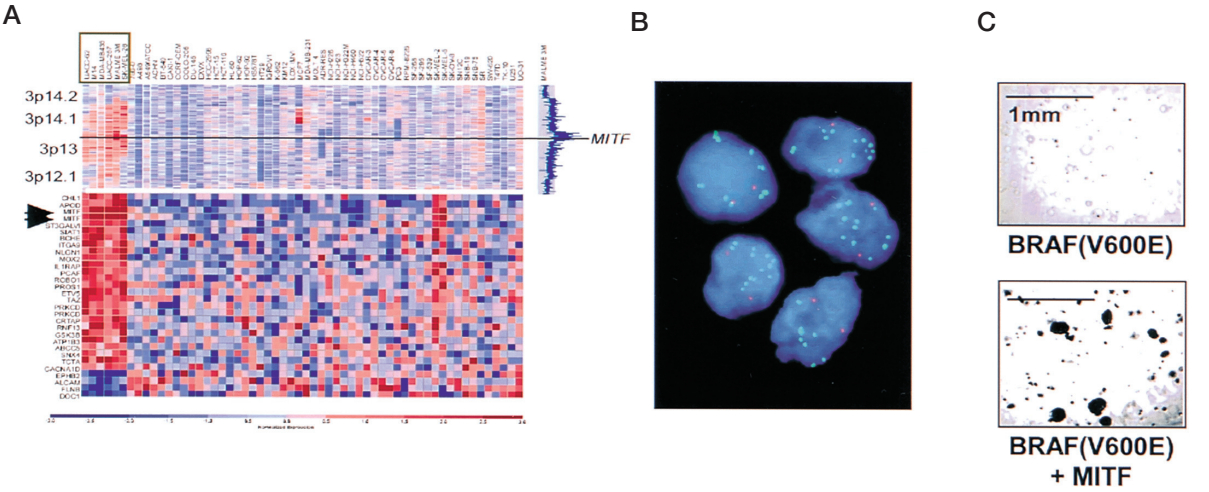

Figure 5. MITF as a lineage survival oncogene in melanoma. (*A*) The region of chromosome 3p amplified in the NCI60 melanoma subcluster was used in a supervised analysis of NCI60 gene expression data (see text for details). (*Top*) Colorgram shows high (*red*) and low (*blue*) copy number at 3p14-3p12; the location of MITF within chromosome 3p is indicated. (*Bottom*) A second colorgram depicts genes from chromosome 3 whose mRNA expression patterns correlated significantly with the 3p amplicon class. Arrows indicate *MITF* probe sets. (*B*) A digoxin-labeled probe (*green*) was used to detect the MITF locus, and a SpectrumOrange control probe (Vysis; *red*) detected the chromosome 3 centromere by FISH in a melanoma tissue microarray. A case of *MITF* amplification is shown. (*C*) Soft agar assays following BRAF(V600E) (*top*) or BRAF(V600E)+MITF retroviral transduction of immortalized melanocytes. Colonies were photographed after 8 weeks (Magnification, 32x). (All panels adapted, with permission, from Garraway et al. 2005 [©Nature Publishing Group].)

Kaplan-Meier analysis. Here, *MITF* amplification associated significantly with adverse patient overall survival. On the tissue array, *MITF* amplification was also associated with significantly increased MITF protein as measured by automated fluorescence analysis (AQUA) (Camp et al. 2003). Together, these data presented compelling genetic evidence that *MITF* might mediate an oncogenic function in melanoma.

We also examined the oncogenicity of MITF directly by ectopic expression within melanocytes that had been immortalized by serial retroviral transduction of telomerase (hTERT), CDK4(R24C) (an INK-resistant variant), and p53DD (a dominant-negative p53 protein) (Garraway et al. 2005). Although retrovirally transduced MITF was not oncogenic in these melanocytes when overexpressed by itself, it transformed these melanocytes in cooperation with the BRAF(V600E) mutant protein, as assayed by growth factor independence and soft agar experiments (Fig. 5C). Conversely, introduction of a dominant-negative MITF construct into melanoma cell lines resulted in growth inhibition. These findings strongly suggested a critical role for MITF in melanoma genesis and survival, particularly in the setting of cell cycle deregulation and excess BRAF-mediated MAP kinase activation.

LINEAGE ADDICTION AND TUMOR DEPENDENCY IN MELANOMA

To a first approximation, the tumor-promoting function of MITF may resemble oncogene addiction: A subset of melanomas exhibits deregulation of MITF (through increased gene dosage or by other mechanisms as yet undetermined), and this deregulation may be essential to melanoma cell survival. However, MITF action differs importantly from oncogene addiction in that it does not seem to involve a specific gain-of-function event that is absent in nontransformed melanocytes. Rather, it may represent the persistence in melanoma of a master survival function also operant in cells of the melanocytic lineage during development and differentiation. In this regard, MITF function constitutes a novel oncogenic mechanism, which we term lineage addiction or lineage survival. In hindsight, other well-characterized oncogenes may provide analogous lineage survival functions in their native cell types, such as the androgen receptor (prostate), FLT3 (myeloid), and cyclin D1 (breast). Thus, lineage addiction may represent a tumor dependency mechanism exploited by many cancer types through lineage-restricted genomic alterations.

In melanoma, this lineage addiction mechanism also pinpoints MITF as a nodal point within a key genetic dependency already recognized to offer therapeutic promise (Fig. 6). Here, the relevant tumor-promoting alteration (MITF amplification) converges on a fundamental lineage survival process, which itself depends crucially on a single cellular pathway centered around—but not restricted to—MITF. Indeed, this dependency is predicted to co-occur with other genetic alterations that activate MAP kinase signaling and inactivate the p16/Rb pathway. Aberrant MAP kinase pathway activation is commonly observed in melanoma, but MAP kinase triggers ERK- and RSK-dependent phosphorylation events that lead to tightly coupled activation and degradation of MITF under normal circumstances (Hemesath et al. 1998; Price et al. 1998; Wu et al. 2000). Presumably, melanomas that exploit a MITF-dependent lineage survival mechanism avert this degradatory MAP kinase effect by deregulating MITF through amplification or other mechanisms. Inactivation of the p16/Rb pathway should also be required because MITF has been shown to induce

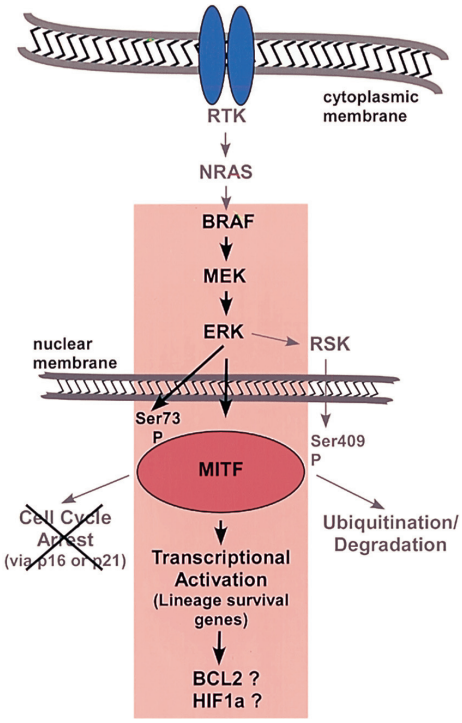

Figure 6. A lineage addiction pathway in melanoma. Deregulation of MITF activity may mediate a critical lineage dependency operant within a subset of melanomas. This dependency may also be characterized by aberrant MAP kinase pathway activation (e.g., through BRAF mutation) and cell cycle deregulation (e.g., through p16/CDK/pRB pathway inactivation). Transcriptional activation of lineage survival genes by MITF (possibly including the antiapoptotic BCL-2 oncogene and the proangiogenic HIF1a gene) may contribute to melanoma genesis and progression.

melanocyte growth arrest when this pathway is intact (Loercher et al. 2005). In support of this model, our data suggest that MITF may require both loss of p16/Rb function and aberrant MAP kinase signaling (e.g., through cooperation with mutated *BRAF*) to act as an oncogene. Moreover, these findings suggest that molecular probes interrogating the MITF locus, MAP kinase pathway activation, and p16/Rb pathway status may prove capable of identifying this dependency in situ at the time of melanoma diagnosis.

To this end, the MAP kinase/MITF dependency is also notable in that several putative effector proteins represent the targets of small molecules at various stages of clinical development. For example, BRAF undergoes an activating V600E mutation in a large percentage of melanomas (Davies et al. 2002); this enhanced activity appears to be required for melanoma cell survival (Hingorani et al. 2003). Accordingly, several companies have developed compounds that inhibit B-Raf or MEK, key MAP kinase effector proteins (Bollag et al. 2003; Sebolt-Leopold 2004; Wilhelm et al. 2004). Transcription factors such as MITF have historically been considered less "drug-able"; however, the MITF target gene BCL-2 has been targeted by antisense compounds in advanced clinical development (Manion and Hockenbery 2003). Loss of p16/Rb pathway regulation, a common melanoma occurrence that may cooperate with the MITF lineage survival mechanism (as described above), leads to increased cyclin D/CDK4 activity and cell cycle progression. Several CDK inhibitors are also in clinical or preclinical development (Dai and Grant 2003; Shapiro 2004); conceivably, these could be implemented along with MAP kinase and/or BCL-2 inhibitors in a combinatorial therapeutic cocktail tailored to the genetic makeup of a defined and clinically identifiable melanoma subtype.

CONCLUSIONS

The success of targeted cancer therapeutics depends heavily on the ability to define molecular tumor subtypes and the salient dependencies underlying their genesis and progression. Tools of the genome era offer tremendous promise in this regard; already, the application of DNA microarray platforms to cancer genome characterization has resulted in the discovery of novel tumor subsets as well as the cancer genes directing their biology. The discovery that MITF acts as a melanoma oncogene constitutes an informative example in this regard; its putative function in melanoma also illustrates a newly recognized lineage addiction mechanism that may prove both operant and therapeutically tractable in other cancer types. The broad application of genomic approaches to characterize human tumors and their counterpart model systems (cell lines, short-term cultures, xenografts, etc.) should enable a productive and integrated avenue that improves biological understanding and therapeutic application.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (L.A.G., M.A.R., D.L.R., and D.E.F.), the American Cancer Society (M.L.M.), the Flight Attendant Medical Research Institute (M.L.M.), the Doris Duke Foundation (D.E.F.), the Tisch Family Foundation (W.R.S.), and the Damon Runyon Cancer Research Foundation (W.R.S.).

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Cold Spring Harbor Symposia on Quantitative Biology

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Cold Spring Harb Symp Quant Biol 2005 70: 25-34
Access the most recent version at doi:10.1101/sqb.2005.70.016