generating permanent transgenic helminths (*15*). By adopting this technology, the assessment of gene function in these parasites—by gene deletion or disruption or by exchanging codons—should be possible. Developing both transgenic parasitic worms and stable cell lines will functionally equip investigators with game-changing tools to keep pace with other, more tractable areas of biology.

For the first time, these resources will enable the field to address fundamental evolutionary, biomedical, and immunological questions that have stymied the development of new treatments. How does immortality in parasitic flatworms operate? That is, how do parasite stem cells contribute to the developmental plasticity involved in the complex life cycles of these animals (including both asexual and sexual components) within obligate hosts? And, in the case of *Echinococcus*, for example, do the highly proliferative asexually reproducing larvae present new opportunities for interventions? Also mysterious is how long-term host-parasite relationships develop and are maintained to the mutual benefit of both symbiotic species. Once infected, helminth-mediated host immunomodulation and host-mediated parasite elimination compete, continuously striving to gain the upper hand. For example, age-dependent immunity is seen in schistosome-infected humans, where children harbor the vast majority of parasites in endemic areas. What are the molecular mechanisms underlying these processes, and what opportunities do they offer for developing new intervention strategies?

Adapting breakthrough biotechnologies to these neglected pathogens hopefully will drive the innovations needed to more fully understand their intriguing biology and pathogenesis. This could open up an exciting new frontier of translational options needed to control the damage caused by these harmful helminths. ■

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ILLUSTRATION: V. ALTOUNIAN/*SCIENCE*

CANCER

Attack of the clones

What makes lung cancer so resilient?

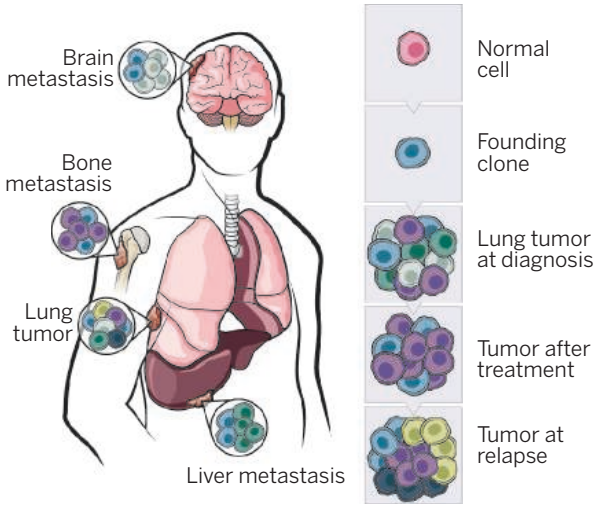
By **Ramaswamy Govindan**[1,2]

Nearly 40 years ago, it was presciently observed (*1*) that each patient's cancer would require individual therapy and that this would be "thwarted" by the emergence of resistant cells. This prediction has proven to be depressingly true in various malignancies. It is now evident that malignant clones and subclones evolve not only through gradual acquisition of mutations but are also secondary to abrupt catastrophic events (such as massive chromosomal rerarrangement), leading to genomic heterogeneity in a tumor over time. The advent of next-generation sequencing has enabled these processes to be studied in unprecedented detail. Considerable intratumoral heterogeneity has been demonstrated in certain hematological malignancies and cancers of the breast, ovary, bladder, prostate, pancreas, and kidney (*2*–*8*). On pages 256 and 251 of this issue, Zhang *et al.* (*9*) and de Bruin *et al.* (*10*), respectively, describe the universal prevalence of intratumoral heterogeneity in lung cancer, with important implications for future research.

Lung cancer is one of the leading causes of cancer-related death globally. Non–small cell lung cancer (NSCLC) is the most common type, accounting for nearly 85% of all newly diagnosed cases. A sizable minority of patients with lung cancer, ranging from 10 to 40% depending on the region of the world, reports no history of tobacco smoking (*11*). More than half report quitting tobacco smoking years before the diagnosis of lung cancer. Most patients with NSCLC either present with metastatic disease or their cancer recurs despite undergoing treatment for seemingly localized disease, underscoring the systemic nature of this disease. Cytotoxic chemotherapy regimens developed over the past few decades have produced only modest improvements in survival in patients with metastatic NSCLC. However, a small subset of patients (15%), with tumors driven by activating mutations in the gene encoding epidermal growth factor receptor (*EGFR*) or rearrangements in the gene coding for anaplastic lymphoma kinase (*ALK*), benefit substantially from specific targeted therapies. Even these patients eventually succumb to tumor progression within a few years of diagnosis. It is critical to understand fully the molecular events leading to the initiation, maintenance, and progression of lung cancer to improve these outcomes.

The complex genomic landscape of NSCLC related to tobacco smoking is characterized by innumerable single-nucleotide variations, gene amplifications, insertions, deletions, and structural rearrangements (*12*, *13*). By contrast, there are strikingly fewer mutations and genomic alterations in the lung cancer specimens from nonsmokers (*14*). Almost all genomic studies in lung cancer reported to date have been conducted with samples obtained from a single region of the tumor, limiting knowledge about the extent of intratumoral heterogeneity and clonal evolution.

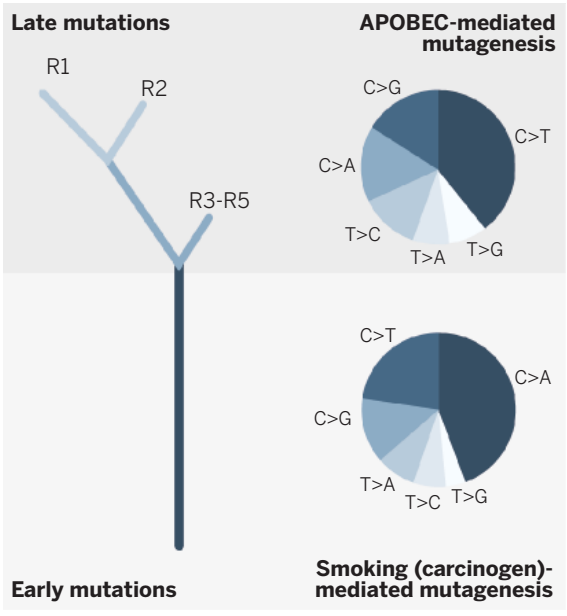
Tumors evolve either in a linear fashion, by acquiring progressively fitter clones that outpace the founding clones, or more com-



Lung cancer resilience. A model is shown of how a tumor may acquire progressively fitter clones, giving rise to subclonal populations and tumor heterogeneity.

[1]*Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA.* [2]*Alvin J. Siteman Cancer Center, Washington University School of Medicine, St. Louis, MO 63110, USA. E-mail: rgovinda@dom.wustl.edu*

monly follow a branched pattern where multiple subclones thrive simultaneously, resulting in a complex heterogeneous tumor (see the first figure). Identification of key "driver" mutations—those that confer a selective growth advantage to a cancer cell—contributing to these branch points in NSCLC is vitally important from the therapeutic standpoint. Zhang *et al.* used multiregion whole-exome sequencing on 11 lung adenocarcinomas whereas de Bruin *et al.* applied whole-exome sequencing and/or whole-genome sequencing on 25 spatially distinct regions from seven localized NSCLC tumor samples. Intratumoral heterogeneity was universal in all the 18 primary tumors. About a third of all nonsilent mutations analyzed by de Bruin *et al.* were present in at least one region but not in all regions of individual tumors. De Bruin *et al.* found evidence of branched evolution with key driver mutations present before, and even after, subclonal diversification, raising the uncomfortable possibility that single-region sampling, as is currently being done in clinical diagnosis, could miss key driver mutations present in specific subclones. By contrast, Zhang *et al.* identified 20 out of 21 known cancer genes in all regions of the individual tumors. As these two studies are too small to allow any definite conclusions to be drawn on this topic, for now, the practice of single-region biopsy should continue for clinical diagnostic purposes. Multiregion, multisite biopsies of metastatic lesions carry considerable risks, and the evidence has to be more persuasive to change this practice.



Clonal evolution. An example of a phylogenetic tree showing the clonal evolution of lung cancer. Pie charts show the spectrum of six types of mutations during early tumor evolution (smoking-induced mutations) and late evolution (APOBEC mediated) (*10*). R indicates regions of the tumor.

More work is needed to understand the prevalence of regionally dominant subclones with known driver mutations and their role in metastases and drug resistance. Zhang *et al.* noted that three patients with a larger fraction of subclonal populations developed recurrent disease after surgery, an observation that deserves further attention with regard to the use of intratumoral heterogeneity itself as a biomarker of poor prognosis.

Two interesting observations made by de Bruin *et al.* suggest the possibility that smoking-related genomic events occur fairly early in the disease, together with early driver events and single-nucleotide variations (see the second figure). Genome-doubling events incorporating characteristic signatures of smoking occurred long before the development of clinical disease in former smokers, suggesting prolonged latency from the first driver events to clinical presentation. Even in the presence of continued tobacco smoking, carcinogen-related genomic events decreased over time along with a concomitant increase in mutagenesis related to activation of a class of enzyme called apolipoprotein B mRNA editing, enzyme-catalytic, polypeptide-like cytidine deaminases (APOBEC).

Clearly, more work needs to be done to understand the clinical implications of clonal evolution and intratumoral heterogeneity. Studies on intratumoral heterogeneity so far have relied mostly on DNA alterations, whereas genomic events contributing to clonal evolution likely extend beyond single-nucleotide variations and copy-number alterations. Multiregion analyses need to incorporate sample procured from metastatic sites along with their corresponding primary lesions to identify the subclones enriched in metastatic foci. In addition, a better understanding of clonal evolution at metastatic sites, perhaps under selective pressures from local tissue environments, could help identify patients at risk for recurrence and distant metastases, and perhaps lead to specific therapies to halt the inexorable spread of cancer to other organs.

The distorted cancer genome landscape is sculpted continually by stochastic events and therapy-induced alterations. Understanding this process requires serial biopsies to characterize clonal evolution both spatially and temporally. Emerging technologies would hopefully facilitate comprehensive genomic analyses of circulating cell-free DNA (so-called liquid biopsies) to obviate the need for invasive core biopsies. Further, statistical methods and algorithms should be developed to elucidate the interplay between dysfunctional gene and protein networks using an integrated multilayered "omics" approach.

To accomplish these goals, clinical trial infrastructure must be optimized to incorporate genomic studies along with interventional clinical studies. For example, the Adjuvant Lung Cancer Enrichment Marker Identification and Sequencing (ALCHEMIST) trial will screen ~8000 patients with surgically resected NSCLC to test for the presence of *EGFR* mutations and *ALK* rearrangements and determine the efficacy of targeted drugs in the postoperative setting. The trial will include studies to identify genomic alterations in NSCLC. Attempts will be made to collect samples at the time of relapse to study clonal evolution following postoperative therapy. A large prospective effort, Tracking NSCLC Evolution Through Therapy (TRACERx), and its sister study, Deciphering Anti-tumor Response and Evolution with Intratumor heterogeneity (DARWIN), will also contribute to understanding intratumoral heterogeneity and clonal evolution.

It is critical to develop a national infrastructure to conduct comprehensive multiregional and multisite genomic studies with samples procured through "warm autopsies" from patients with a wide variety of malignancies. An organized program could collect a large amount of highly informative samples in a relatively short period. Developing new cancer therapies may still be a long and arduous process. A better understanding of genomic alterations is key to develop more rational and effective therapies. ■

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