Halting harmful helminths
Karl F. Hoffmann *et al.*
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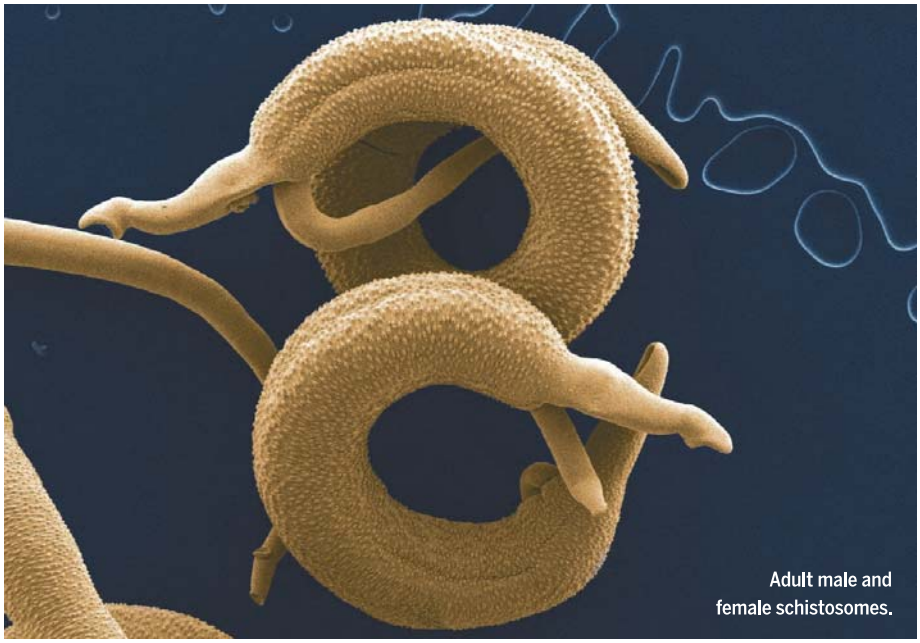

Adult male and female schistosomes.

MEDICINE

Halting harmful helminths

Vaccines and new drugs are needed to combat parasitic worm infections

By **Karl F. Hoffmann,[1] Paul J. Brindley,[2] Matthew Berriman[3]**

More than 300 million people are infected each year with parasitic flatworms such as hydatid tapeworms and blood fluke schistosomes. The diseases caused by such parasitic helminths, including alveolar/cystic echinococcosis and hepatosplenic/urogenital schistosomiasis, are typically chronic but frequently deadly. They are among the 17 neglected tropical diseases listed by the United Nations World Health Organization, and infections by these flatworm pathogens cause ~4 million disability-adjusted life years to be lost annually, although this vastly underestimates the true impact that such long-term and chronic illnesses can have (*1*). Historically considered restricted to the tropics and subtropics, suitable habitats for transmission of these parasites are now expanding into Europe (*2*), and conditions are right for similar expansions to other continents (*3*, *4*). The lack of vaccines perpetuates the unsustainable over-reliance on single-drug chemotherapies, a potentially catastrophic situation unless new solutions are found.

To accelerate the discovery of vaccines, drugs, and exploitable insights into pathogenesis, there has been a drive to apply state-of-the-art technologies to parasitic helminth research. With mammals, great strides have been made in these arenas by harnessing technologies in stem cell research for large-scale mutagenesis studies (*5*). More recently, there has been a shift to develop cell-focused approaches coupled with genome editing (*6*). These strategies—especially the bacterial clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 endonuclease system—have revolutionized the way in which genetic-mutational-phenotypic analyses in eukaryotic species are performed. By contrast, comparable research on helminths has lagged far behind. The difficulty in maintaining parasitic flatworms in the laboratory due to their complex life cycles (typically alternating between two obligate hosts) and their recalcitrance to genetic and cellular manipulations present major bottlenecks for adapting technologies.

Despite these hurdles, progress in the areas of schistosome (blood fluke) transgenesis (*7*), organ isolation (*8*), and stem cell characterization (*9*), coupled with advances in echinococcal (hydatid tapeworm) primary cell cultures (*10*, *11*), portends a transformation in the ability to manipulate and study parasitic flatworms. As a prelude to whole organism or cellular transgenesis, techniques have been honed over the past 15 years to perform loss-of-function studies in schistosomes using posttranscriptional gene silencing (*12*). However, this approach is only transient, often not fully penetrant, and difficult to translate to other parasitic flatworms. Gain-of-function manipulation, based on overexpressing transgenes in schistosomes with plasmid-based vectors, is possible (*13*), but suffers from the same challenges as post-transcriptional gene silencing.

A breakthrough in developing a stable system for manipulating gene function came recently with the discovery that pseudotyped retroviruses (murine leukemia viruses) and, to a lesser degree, transposons (*piggyBac*) can introduce transgenes into all eight schistosome chromosomes, including those found in germ cells (*7*). Translating this to other parasitic flatworms, as well as applying methods for selective targeting of transgene genome integration, should spur investigation of individual gene function.

Coincident with these developments in transgenesis are innovations in parasitic flatworm cell culture. Attention has focused on stem cells (also called germinative cells or neoblasts) and the tissues in which they reside because these cells self-replicate as well as differentiate and, hence, endow the evolutionary and developmental plasticity of parasitic flatworms. It is now feasible to isolate gonads enriched in stem cells (*8*), identify somatic proliferating stem cells (*9*), and initiate limited-passage cell cultures from schistosomes (*14*). Also promising are advances in cell cultivation systems for echinococcal tapeworms, where continuous cultures enriched for replicative and differentiating stem cells can be derived from *Echinococcus multilocularis* (*10*) and "immortal" cell lines can be produced from the closely related species *E. granulosus* (*11*). Although still being refined, it is clear that immortalized cell lines based on the proliferation and differentiation capacity of flatworm stem cells will alleviate the need for maintaining complex parasitic life cycles.

By applying genome editing and knowledge about cell biology and cell culture to blood flukes and hydatid tapeworms, the aspiration of developing stable transgenic parasitic helminths and manipulable cell lines derived from these pathogens is now a near-future certainty. Retroviruses engineered to deliver CRISPR-Cas9-RNA cargos represent the most feasible approach in

[1]*Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Aberystwyth SY23 3DA, UK.* [2]*Department of Microbiology, Immunology and Tropical Medicine and Research Center for Neglected Diseases of Poverty, School of Medicine and Health Sciences, George Washington University, Washington, DC 20037, USA.* [3]*Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK. E-mail: krh@aber.ac.uk*

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?

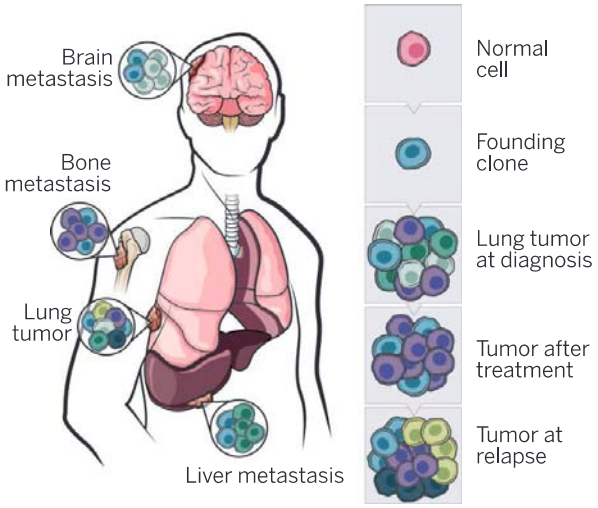
By **Ramaswamy Govindan**[1,2]

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*