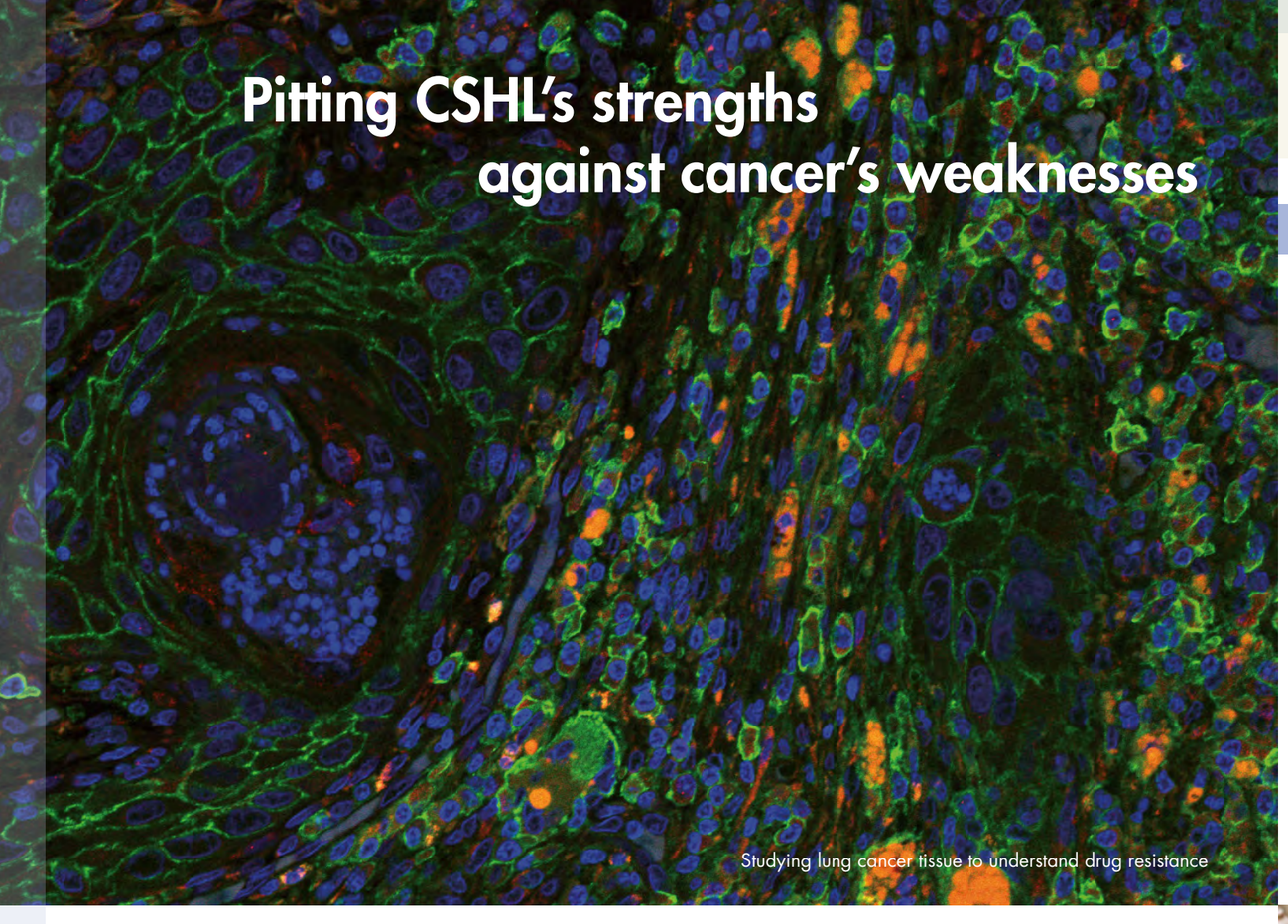
Pitting CSHL's strengths against cancer's weaknesses



Studying lung cancer tissue to understand drug resistance

Cancer research is making a pivotal turn. At Cold Spring Harbor Laboratory, new ways of analyzing the vulnerabilities of cancer cells, new knowledge about the mechanisms of drug resistance, and new approaches to discovering therapeutic targets and rapidly testing their effectiveness *in vivo* are vitally enhancing our ability to confront the disease. Here, on Laboratory grounds, where so many consequential cancer discoveries have been made since James D. Watson made the illness a primary focus of the scientific program in 1968, the "war" on cancer now enters a potentially climactic stage.

The great strengths of CSHL's cancer program, built up steadily over the years, are being integrated with innovative technologies and approaches in President Bruce Stillman's new Cancer Therapeutics Initiative. The Initiative aims to rapidly identify novel therapeutic targets and validate them in mouse models that closely mimic the behavior of specific human cancers. In addition to offering insights into cancer's molecular mechanisms, the preclinical testing of drug candidates within living animals — an important aspect of the Initiative — allows researchers to evaluate cancer's response to new therapies and glean information that can be used to modify treatment strategies and increase the number of drugs that enter the clinic, and, it is hoped, the rate at which they succeed.

A leader in cancer research for four decades, CSHL has been a National Cancer Institute-designated cancer research center since 1987. Now at a point of unprecedented technological and intellectual maturity, the Laboratory's cancer program is playing a leading role in transforming cancer treatment from its long "hit-or-miss" phase that relied on toxic chemotherapies and radiation treatments that kill cells indiscriminately, causing massive and often fatal side effects, to a new stage likely to be characterized by ensembles of targeted therapies tailored to reflect genomic and epigenetic complexities of individual tumors. We sample below only a fraction of the research efforts under way in the laboratories of CSHL principal investigators to address major cancer types: breast, prostate, ovarian, cervical, leukemia, lymphoma, melanoma, lung, liver, brain and pancreatic.

BREAST CANCER:

Battling on multiple fronts

One in eight American women will develop breast cancer and about 90% of those diagnosed will survive at least five years. Several CSHL research groups are working to improve these odds by developing technologies to better define individual risk for this cancer; to detect it at early stages, when it is most treatable; to find out more about its basic causes and cellular origins; and to use genomic data to guide and improve its treatment.

Knowing whether an individual is susceptible to breast cancer is the first step toward saving a life. CSHL quantitative biologists led by Mickey Atwal are developing algorithms — computer-driven mathematical procedures — that hunt for genetic variants that increase breast cancer risk. These methods previously helped link genetic variations in two human genes, the cancer-causing *MDM4* and the tumor suppressor *TSC1*, to increased risk for breast cancer among Northern European and Ashkenazi Jewish women. The team's current search is within a genetic network controlled by the powerful tumor suppressor gene *p53*, which is mutated in more than 50% of all cancer patients.

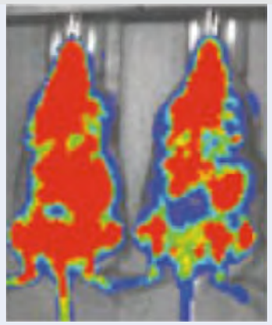
In an effort to provide breast cancer patients and doctors with actionable information that can direct treatment decisions and reduce the risks inherent in a trial-and-error approach to therapy, CSHL scientists led by Michael Wigler and Jim Hicks have developed a "DNA biopsy." This is a diagnostic test that aims to distinguish between cancers that are likely to spread and therefore should receive aggressive treatment, and cancers that are benign and therefore should not be treated. As normal breast cells develop into tumor cells, they accumulate chromosomal rearrangements — structural alterations to DNA that increase or decrease the copy numbers of genes. (Most human cells should have two copies of each gene — one inherited from each parent.) To date, the CSHL team has analyzed copy-number changes in over 1,000 patients and identified three distinct DNA profiles associated with different outcomes. Their new diagnostic test, which is included in clinical trials being carried out at Yale Medical Center and Memorial Sloan-Kettering Cancer Center, should yield critical information about which patients are most likely to benefit from treatment with specific drugs.

The team has also developed another powerful technique, one that can identify dangerous tumor cells from very small samples (such as those obtained via fine-needle biopsy) based on chromosome rearrangement patterns. Called single nucleus sequencing (SNS), this technique reveals the order in which genetic changes occur as tumors develop. Such mutational patterns are now being analyzed to identify changes that signal impending metastasis and to find markers that can predict which breast cancers will respond to specific therapies. Wigler, Hicks and colleagues are now trying to make SNS less costly and scale it up to profile the genomes of thousands of cells at the same time. Such an advance will make it feasible for SNS to be used as a monitoring tool for the first signs of cancer by looking for cancer cells in blood and learning more from biopsies to increase treatment efficiency.

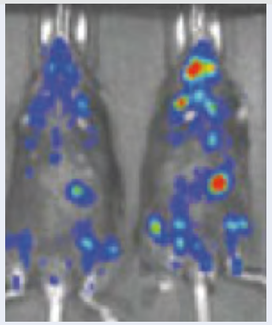
Breast tumors are abnormally organized tissues that contain tumor cells but also supporting cells such as white bloods cells and fibroblasts, and proteins such as collagen. Communication among these different components influences how fast the tumor develops, the likelihood that it will spread to distant organs and its response to therapy. A microscopic imaging method developed by Mikala Egeblad's group allows researchers to watch the interactions between cancer cells and supporting cells in real time in mice with breast cancer as they are being treated with drugs. From the movies thus obtained, the team is learning how these interactions can cause drug resistance, and whether blocking specific interactions between cancer cells and the surrounding tissue will improve sensitivity to drugs.

In a systematic approach to identify genetic factors that make breast cancer cells either vulnerable or resistant to therapy, scientists in Greg Hannon's group are using

WITHOUT TREATMENT



TREATED WITH JQ1



When treated with daily injections of the new drug candidate JQ1, mice with acute myeloid leukemia show fewer signs of disease (red spots) than their untreated counterparts.

RNA interference (RNAi)-based tools that they developed to analyze 100 human cell lines representing a broad spectrum of breast cancers such as, for example, those that are stimulated by estrogen or those that overproduce the HER2 protein. These tools, which the team has made available to the entire breast cancer research community, are also being used to learn more about the genetic characteristics of breast cancer-initiating mammary stem cells, and to find ways of depleting them using microRNAs — a type of small RNA that modulates the activity of genes.

PROSTATE CANCER:

Identifying and treating the serious cases, early

In most cases, prostate cancer is slow-growing and generates few symptoms. Yet 1 case in 100 will rapidly take a lethal course if not treated aggressively. CSHL scientists are developing means of reliably predicting, as early as possible, which cases fall into the urgent category. W. Richard McCombie is involved in a project with collaborators at Memorial-Sloan Kettering Cancer Center (MSKCC) in which the molecular profiles of circulating tumor cells (CTCs) are being determined. Isolated from circulating blood, CTCs potentially contain telltale biomarkers that could help doctors parse patients with treatment-resistant tumors from others, on the basis of a simple blood test. Also in collaboration with MSKCC, Michael Wigler and Jim Hicks are applying their powerful single-cell sequencing method to both CTCs and patient biopsies, to identify prostate cancer biomarkers to guide treatment. Greg Hannon and MSKCC's Charles Sawyers, a CSHL Scientific Trustee, are developing better mouse models of resistant human disease, aiming to obtain a better knowledge of the factors that determine response to therapy.

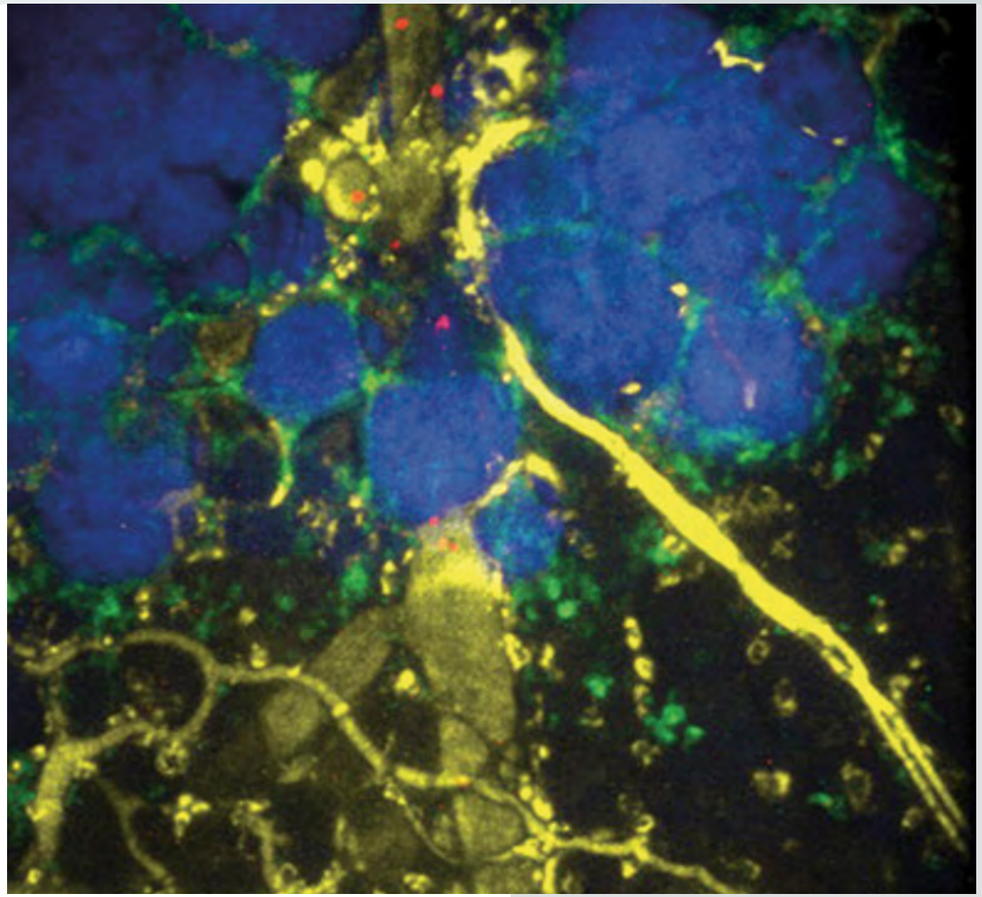
Lloyd Trotman is taking another path toward rapid clinical identification of lethal forms of prostate cancer. He has solved a complex calculus accounting for how the loss of one or two copies of a tumor suppressor gene called *PTEN* either results in prostate cancer or senescence, a state of growth-arrest in which oncogenesis is halted. Recently Trotman has added new permutations to the equation. These involve situations in which prostate cells lose another tumor suppressor, called *PHLPP*, which can act in concert with loss of *PTEN* to produce cancer. He raises the prospect that by monitoring the extent to which the *PTEN* and *PHLPP* genes malfunction, it may be possible to accurately predict whether patients following surgery are on a course to relapse. Checking a patient's "*PHLPP* status" may also help doctors decide on optimal drug treatment strategies, and in the design of more efficient clinical trials.

LEUKEMIA, LYMPHOMA and MELANOMA:

Staging epigenetic interventions

Traditionally seen as a disease caused by genetic changes, cancer is now known also to involve epigenetic changes, which modify the way genes are expressed without altering the DNA code itself. Unlike genetic mutations, disruptions in epigenetic machinery — which can transform normal cells into cancer — are potentially reversible. So epigenetic therapy is now being intensely pursued as one of the most promising anti-cancer strategies. Four FDA-approved epigenetic drugs are already in use.

CSHL scientists hope to add more to the list, starting with Christopher Vakoc, who earlier this year used a novel RNAi-based strategy to discover an epigenetic target for acute myeloid leukemia (AML), an aggressive cancer that is incurable in 70% of patients. This target, a protein called BRD4, allows AML cells to divide in an uncontrolled fashion. By blocking BRD4 with a first-of-its-kind chemical inhibitor, JQ1, developed by collaborator James Bradner of Dana Farber Cancer Center, Vakoc's team was able to suppress aggressive AML in experimental models. With JQ1 expected to enter phase I clinical trials within two years, the collaborators are now pre-clinically developing and optimizing similar drug targets for different genetic subtypes of AML. Because JQ1 and its chemical brethren work by choking off a common cancer-fueling pathway, the hope is that these drugs will work on other forms of cancer as well. The highly successful strategy employed by Vakoc to discover BRD4 is now being adapted to search for therapeutic targets in lymphoma and melanoma.

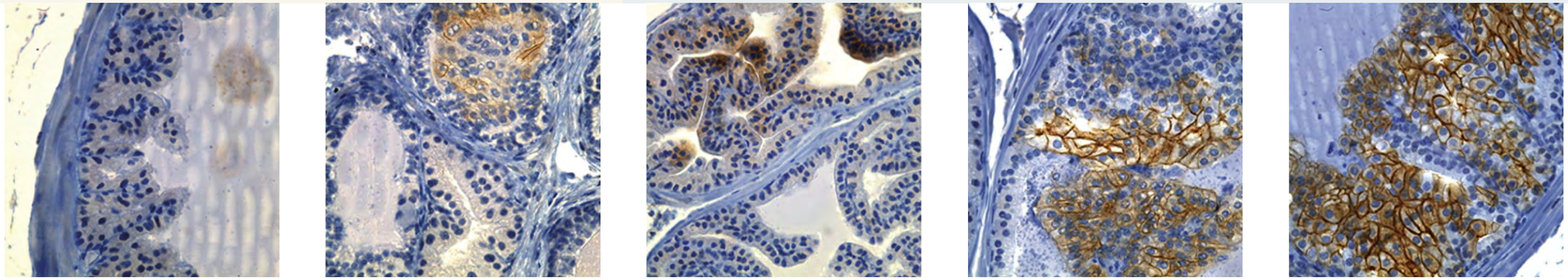


Four-color, real-time imaging of a mouse breast carcinoma shows a tumor with cancer cells (blue), its network of blood vessels (yellow) and two different types of immune cells (red and green).

LIVER CANCER:

New options for targeted therapy

The recent discovery of a therapeutic target, a gene called *FGF19*, by Scott Powers' group, spells hope for more effective liver cancer treatments, which are currently few in number and typically ineffective. An antibody that halts liver tumor growth by blocking the activity of the protein produced by the *FGF19* gene is currently in preclinical development at the pharmaceutical company Genentech. Treatment with this antibody might benefit patients who carry multiple copies of *FGF19*. Powers' group is now pursuing new therapeutic targets and diagnostic markers by examining cancer cells' altered epigenetic landscape, in particular, changes in their pattern of DNA methylation — the tagging of DNA



Prostate tissue samples of five mice, all 8 months old, showing effect of loss of the tumor suppressors *PTEN* and *PHLPP1* in various combinations. Compared with the healthy mouse (far left), the others, moving toward the right, show progressively more pervasive activation of oncogenetic factors (brownish-yellow structures), reflecting increase in genetic damage due to loss of only 1 *PTEN* gene copy (2nd from left) to loss of 1 *PTEN* and 2 *PHLPP1* gene copies (far right).

sequences by methyl molecules. Early data hints at promising candidates within a subset of genes that confer "stemness," or the ability to self-renew — a quality that is repressed in normal adult liver cells.

OVARIAN and CERVICAL CANCER:

Reckoning with cellular and viral culprits

Scott Powers and Alex Krasnitz are translating genomic information generated by the NCI's Cancer Genome Project and other rich data sources into new therapeutic targets for ovarian cancer. As investigators in the NCI's Cancer Target Discovery and Development Network (CTD2), they have analyzed the genomes of over 500 ovarian cancer samples and found "an amazing degree of [gene] copy-number instability." Krasnitz is using "*in silico* cancer genomics" — advanced computational methods of detecting significant patterns — to understand the mountain of tumor data. These have already led to the identification of genome regions harboring recurrent copy-number changes. Over 100 candidate genes have been culled from these regions and are being tested by Powers' team in mouse models of human cancer for their suitability as drug targets. Robert Lucito and Nicholas Tonks of CSHL and others at MSKCC are also integrating genomics and epigenomics to find new ovarian cancer oncogenes and tumor suppressors, whose functional networks are being analyzed with sophisticated computer programs. Separately, Lucito has identified a gene, *CHD3*, whose epigenetic silencing coincides with resistance in ovarian malignancies to the chemotherapy drugs carboplatin and cisplatin. *CHD3* could be a diagnostic marker and future drug target.

Arne Stenlund and Leemor Joshua-Tor have obtained a detailed understanding of the processes that enable papillomavirus (HPV), the virus that causes malignant cervical cancer, to replicate and proliferate. Stenlund hopes to help develop a small-molecule drug that can inhibit HPVs, to help women who don't receive a preventive vaccine and those who are already infected.

LUNG CANCER:

Getting to the bottom of drug resistance

Researchers have puzzled over the confounding ability of cancer cells to develop resistance even to the most powerful targeted therapies. In non-small cell lung cancer (NSCLC), erlotinib, marketed as Tarceva, has provided periods of remission for a subset of patients with a particular mutation, in the gene encoding a cellular receptor called EGFR. Disappointingly, however, nearly all who respond suffer relapses and die within a year or two. Raffaella Sordella's lab, which is working on this problem, has conducted experiments suggesting that some tumor cells may be intrinsically resistant to erlotinib. These cells tend to exhibit features of a transformation called EMT, in which cancerous but non-metastatic epithelial cells that line the lung take on characteristics of mesenchymal cells, which tend to be pro-metastatic. Perhaps the most intriguing quality of the seemingly resistant cells is that they secrete elevated amounts of IL-6, a signaling molecule known to increase inflammation. Clinical trials are under way in which erlotinib is co-administered with antibodies blocking IL-6 to find out whether removing IL-6 from the tumor microenvironment might increase cancer cells' sensitivity to erlotinib.

In other work, Sordella is collaborating with Adrian Krainer to explore the possibility that a process called alternative splicing, in a gene encoding the master tumor suppressor p53, may be involved in a process in which lung cells are prevented from entering a protective state called senescence and instead become cancerous. Sordella is also joining forces with Darryl Pappin of CSHL and Brendon Stiles of Weill Cornell Medical College in a search for biomarkers in NSCLC.

BRAIN CANCER:

Inducing cells to differentiate rather than proliferate

Glioblastoma is the most common form of brain cancer and very difficult to treat. No wonder: malignant glioma cells are notorious for their genetic complexity and heterogeneity; most harbor myriad genetic and epigenetic alterations, which drive tumor initiation and progression. Hongwu Zheng is taking a bold path toward a new form of treatment. In a mouse model of human glioblastoma, his team seeks to force malignant cells to undergo terminal differentiation, hoping in this way to eliminate or reduce the cancer, rather than try to kill the malignant cells outright with toxic chemotherapies.

Medulloblatoma is much rarer, but it's the most prevalent brain cancer afflicting children, and a leading cause of pediatric death. It is thought to begin with the failure of stem-like cerebellar granule cell precursors, or GCPs, to differentiate. Linda Van Aelst and collaborator Mary E. Hatten at Rockefeller University are studying the signaling pathways and extracellular cues that set GCPs on a proper developmental path. This approach has implicated a class of signaling molecules called Rho GTPases, one of which has been found to play a critical role in controlling whether GCPs proliferate or differentiate. They are testing this molecule, DOCK7, in a mouse model of medulloblastoma, while continuing to study how "Rho regulators" exert their effects — important information for future drug development.

PANCREATIC CANCER:

Turning the tables on poor prognosis

A 6-month mean survival time makes cancers of the pancreas among the most lethal. CSHL investigators are obtaining fundamental scientific insights about what distinguishes these lesions, as a basis for effective treatments. Greg Hannon's team studies the role of small RNAs as oncogenes and tumor suppressors and seeks to exploit RNAi libraries they have developed to identify new therapeutic targets. Mikala Egeblad is using advanced microscopy to learn how tumors are affected by surrounding stromal tissue. Robert Lucito looks closely at the genetic irregularities in tumors, and has dissected the mechanism by which overexpresson of a gene called *PAK4* is implicated in the emergence of cancer.

Hema Bashyam and Peter Tarr