

National Cancer Institute

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CCR connections

CENTER FOR CANCER RESEARCH

ccr.cancer.gov

Risk Variants

Tracing the Causes of Kidney Disease

U.S. DEPARTMENT
OF HEALTH AND
HUMAN SERVICES

National Institutes
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Center for Cancer Research

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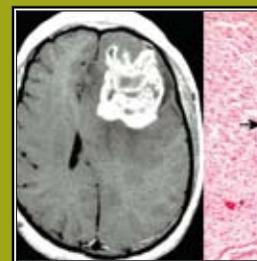
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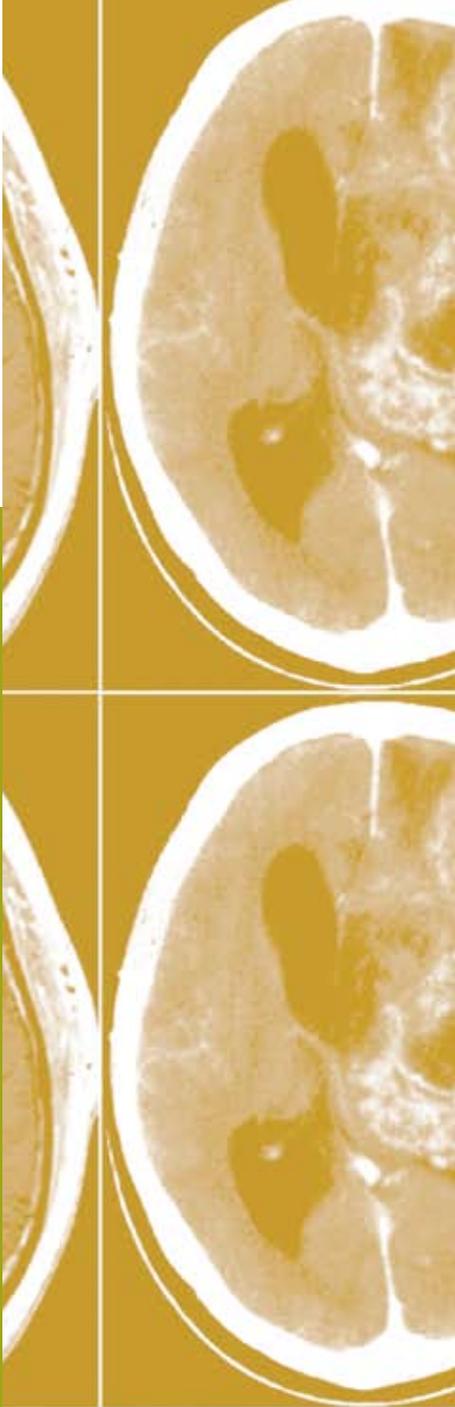
IN THE CLINIC



Radiating Change

The mission of CCR is:

To inform and empower the entire cancer research community by making breakthrough discoveries in basic and clinical cancer research and by developing them into novel therapeutic interventions for adults and children afflicted with cancer or infected with HIV.



<http://home.ccr.cancer.gov/connections/>

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The Strengths of a Diversified Portfolio

Managing an investment portfolio is no easy task. As the current economic climate attests, steady returns depend on both the quality and the diversity of investment opportunities. Supporting biomedical research is a different kind of investment—one in which the return is not measured in dollars but, ultimately, in disease prevented or cured—however, it too requires a diversified portfolio strategy. Biomedical science progresses through a combination of steady accrual of basic information, unpredictable technical or conceptual breakthroughs, and the means to translate new knowledge into clinical action. In this issue of CCR connections, we see strong examples of our research investment that span the ranges of low to high risk and short- to long-term returns.

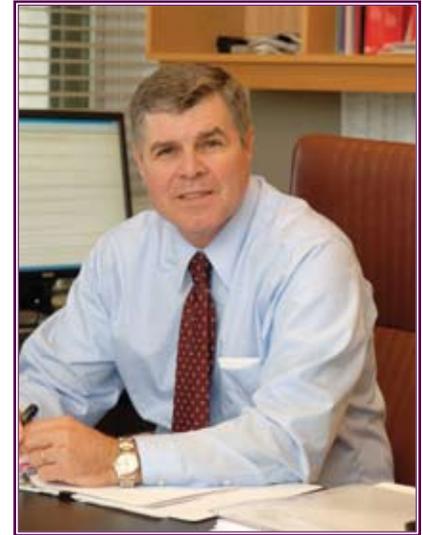
Why would an institute committed to developing cures for cancer and AIDS invest research dollars in studying how yeast mate or how mice form their tails? The answer, as discussed in two feature articles (“Balancing Silence: How a Cell’s Fate Is Determined” and “Cancer Research Takes Flight: *Wnt* Signaling in Development and Disease”), is that such studies address the fundamental mechanisms of cell development and fate that turn against us when cancers form. We may not always know at the outset how far off a clinical payoff might be and what form it will take, but we know that steady investment in basic research is the only way to break new ground.

The Center for Cancer Research (CCR), of course, also invests heavily in research questions aimed squarely at cancers and capitalizes on its rare position as an institute that has benches and bedsides in the same buildings. Several investigators featured in this issue have seen their work go from inhibiting cancerous proliferation in a dish to first-in-man studies (see

“Going after the Real Killer: Metastatic Cancer” and “Radiating Change”). This work represents the kind of translational research for rare and difficult-to-study cancers that the pharmaceutical and biotechnology sectors lack the economic incentives to tackle, but has clear returns in terms of individual lives.

The goal of overcoming economic disincentives to cure disease can result in unusual research tactics. In “By Land or by Sea: High-Yield Harvesting of an Anti-HIV Protein,” we learn of work to produce large quantities of a recently discovered HIV antiviral by infecting tobacco plants with a virus carrying the gene to produce it. As the article points out, the problem of local production and distribution of life-saving drugs to developing countries is a research problem as important as discovering such drugs to begin with and has the potential to impact millions.

In making often difficult decisions on how best to deploy our finite budget and resources to maximize the impact of our scientists and their discoveries, we



(Photo: B. Branson)

Robert H. Wiltrout, Ph.D.

realize that all of our research investments are not going to have immediate payoffs. We also realize that some will rely on unpredictable parallel advances in other fields, as when virology research benefits from new discoveries in molecular biology to impact cancer (see “Keeping Oncogenic HPV in Check: How the Interplay between HPV Oncoproteins and microRNAs Affects Carcinogenesis”). We even know that some very high-risk projects may not succeed at all. Our job, as is any good portfolio manager’s, is to ensure that the research we fund is both broad and deep enough to produce a steady flow of new discoveries that improve human health.

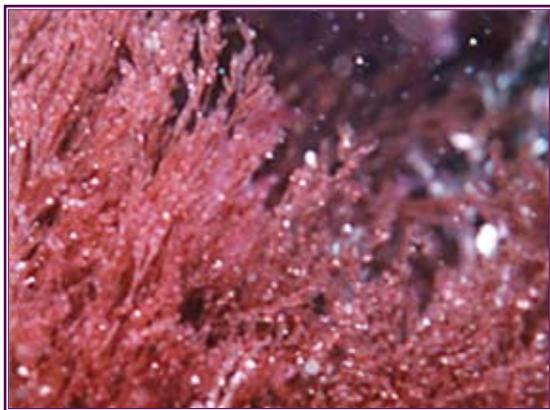
Robert Wiltrout, Ph.D.

Director, Center for Cancer Research

By Land or by Sea:

High-Yield Production of a Marine Anti-HIV Protein in Plants

Photo: D. Newman, NCI Natural Products Repository



Red algae *Griffithsia*, found off the coast of New Zealand, from which the HIV entry inhibitor griffithsin was isolated.

Although biomedical research has led to enormous progress in the prevention and treatment of disease, many would agree that developing countries have not yet reaped proportionate benefits, remaining caught in a cycle of poverty and disease. Drug development aimed specifically at providing medicines to those living in resource-poor areas has its own challenges—especially for the cost-effective production and widespread distribution of antiretroviral therapies to those living with HIV/AIDS.

In 2005, Barry O’Keefe, Ph.D., Associate Scientist in the Molecular Targets Development Program (MTDP) at CCR, and colleagues identified an antiviral protein that they named griffithsin (GRFT) after the red algae from which it was isolated. The research, performed in the MTDP, originated from a marine extract in NCI’s Natural Product Extract Repository (see “The Natural Products Repository: A National Drug Development Resource” in Vol. 2, No. 2 of *CCR connections*), which has collected hundreds of thousands of natural product extracts from around the world.

The ability of GRFT to restrict HIV entry into cells in quantities measured at a trillionth of a gram made it exponentially more potent than other inhibitors studied. The researchers licensed GRFT for prophylactic use against HIV but found that producing this “biologic” drug by the standard method of engineering *E. coli* bacteria to produce it did not yield large enough quantities of GRFT for use as a topical microbicide. So they set out to find a more cost-effective and higher-yield process of manufacturing the protein. Working with collaborator Kenneth Palmer,

Ph.D., and colleagues at the University of Louisville, the team found a solution.

In the April 14, 2009 issue of *The Proceedings of the National Academy of Sciences*, the researchers announced a breakthrough in the scalable manufacture of GRFT using a plant closely related to tobacco, *Nicotiana benthamiana*. They engineered the GRFT gene into tobacco mosaic virus, which they then used to infect the tobacco plants. Once the viral genes integrated into their hosts, the plants produced griffithsin (called GRFT-P). Twelve days after infection, harvest plants yielded about a gram of GRFT-P per plant. The researchers then developed a simple three-step purification process that produced about 99.5 percent pure material.

When tested against a panel of five antibody-based inhibitors of HIV entry, GRFT-P was shown to be effective against all three dominant clades of the virus, whereas each of the antibodies showed inactivity against a certain clade. “And even the most resistant strain to GRFT-P was still more sensitive than the most sensitive strain to any of the other agents,” said Dr. O’Keefe. “The potency is

really through the roof.” In addition, the researchers conducted safety and efficacy studies in animal models and human cervical explants, both with positive results. GRFT-P is also an extremely stable protein and can be shipped to many areas at room temperature without the need for refrigeration, a key advantage in resource-poor areas. It is currently being formulated into small sheets of film that women can use discreetly as an HIV control microbicide. “It’s amazing because you started with something from the ocean and then took it through bacteria, through a virus, and then to one of the oldest medicinally used plants—tobacco—which is now making something that fights HIV,” said Dr. O’Keefe. “And because it’s so temperature stable and such a robust protein, conceivably you can have something like that in a little foil packet on a street corner in Zimbabwe.”

To learn more about Dr. O’Keefe’s research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=okeefe>.

Control Your Cell Fate:

How the Mammary Microenvironment Redirects the Fate of Stem Cells

No cancer cell is an island; carcinogenesis does not occur in isolation. Rather, the cellular interactions and cross talk that take place within its microscopic ecosystem help to drive the uncontrolled growth that results in tumor formation. The development and progression of every cell's fate—normal or cancerous—is shaped by the chemical signals released by the multitudes of other cells that lie in its neighborhood: its microenvironment. It follows, therefore, that modulation of this microenvironment can alter cellular differentiation.

Several studies have demonstrated the dominance of the cellular niche over stem cells during normal development, showing that cell fate can be redirected across lineage boundaries in various models. In the September 2008 issue of *The Proceedings of the National Academy of Sciences*, Gilbert Smith, Ph.D., Senior Investigator and Head of the Mammary Stem Cell Biology Section at CCR, and his colleagues further illuminate how tissue-specific signals of differentiated somatic cells alter adult stem cell fates. Specifically, they show that neural stem cells (NSCs) can be reprogrammed into mammary epithelial-cell lineages simply by mixing mammary epithelial cells (MECs) in the mammary fat pad.

In a study published a year previously, Dr. Smith and colleagues

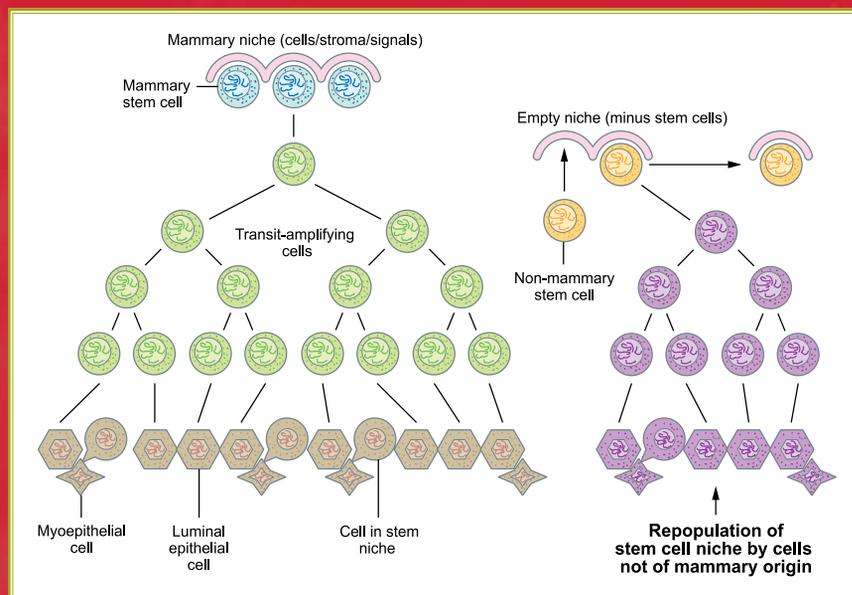
demonstrated that cells isolated from a mature testis, when mixed with normal MECs in the context of a mammary fat pad, cooperated with these cells and contributed progeny to normal mammary epithelial outgrowth and normal mammary function. But because the testicular cells were comprised of about 10 percent germinal stem cells and 20 percent Sertoli cells, with the remainder of the cells from spermatozoa lineage, the researchers were unable to distinguish which cells were being reprogrammed. To overcome this limitation, the researchers turned to isolated NSCs that could be maintained *in vitro*.

The researchers found that the purified cell population could be reprogrammed successfully using the same protocols as in their previous

study. The bona fide NSCs could be reprogrammed into multipotent MECs with the capacity to produce progeny that differentiate into secretory or myoepithelial cells. This indicates that cellular signals from the mammary microenvironment were capable of redirecting the NSCs to form mammary cells. The team is currently engaged in studies to understand how these microenvironmental signals direct mammary cell growth and how those signals might be challenged to control the overproduction of mammary epithelial cells that results in breast cancer.

"Recently, we have extended our studies to include cancer cells; they have been shown by earlier investigators to be responsive to normal developmental environments," said Dr. Smith. "A way to think about cancer is as a developing tissue where the microenvironment of the tumor promotes tumor expansion, development, and growth... So if cancer cells can respond normally to a non-tumor microenvironment, it might be possible to determine what factors might control the growth and expansion of cancer *in situ*." This work points to a promising new direction for therapeutic research. Modulation of the cellular microenvironment to redirect cellular differentiation pathways may one day be used to "normalize" malignant cancer cells.

To learn more about Dr. Smith's research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=gsmith>.



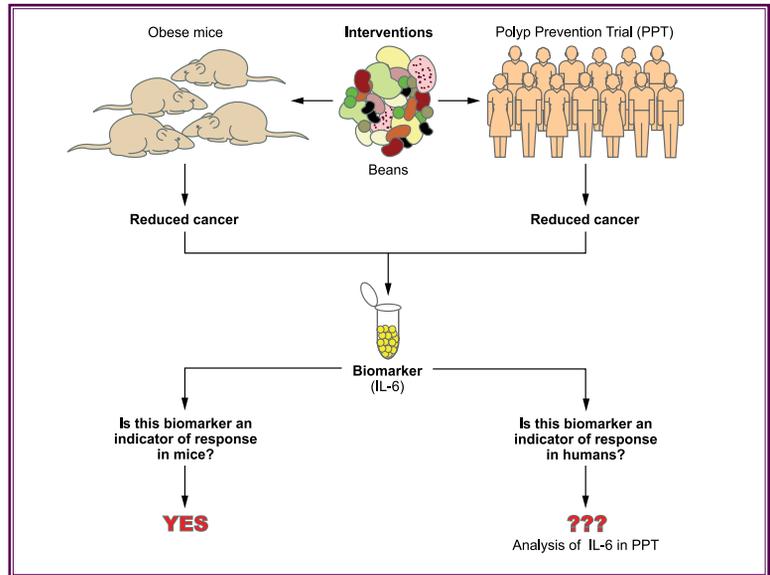
(Image: J. Kelly)

Cellular signals from the mammary microenvironment redirect the fates of non-mammary stem cells.

A Diet That Works:

New Study Shows Early Response of Colon Cancer to Dietary Change

Lifestyle plays an important role in human health, and conscious choices such as adopting healthier dietary habits have become especially crucial in 21st century healthcare as one means to reduce spiraling costs. Growing support exists to better understand diet and disease relationships. But setting up a trial to test a dietary intervention is often challenging since it is difficult to measure how well the intervention is working before actual disease onset.



(Image: J. Kelly)

Discoveries from the Polyp Prevention Trial suggested that a diet rich in beans would reduce the risk of colon cancer. Follow-up studies in mice proposed interleukin-6 (IL-6) as a biomarker of this response. Will IL-6 levels also reflect the efficacy of dietary intervention in humans?

In one of the first studies of its kind, Nancy Colburn, Ph.D., Chief of the Laboratory of Cancer Prevention at CCR, published research in the January 2009 issue of *Cancer Prevention Research* that identified biomarkers of early response to an efficacious dietary intervention—whole navy beans and bean extracts—for reducing the development of colon cancer in genetically obese mice.

In a previous study, Dr. Colburn and colleagues designed an experiment based on what had been observed in the Polyp Prevention Trial (PPT)—a human study that set out to determine whether or not a diet high in fruits and vegetables could reduce the recurrence of colon polyps (abnormal, potentially cancerous tissue growth) in at-risk individuals. The results of that trial revealed that those who ate the highest amount of beans showed only a one-third recurrence rate. Taking this information, Dr. Colburn designed a study using genetically obese mice injected with azoxymethane to induce colon carcinogenesis, placed them on a navy bean diet (whole bean, bean residue fraction, or bean extract fraction), and

found reduced tumor growth in all three groups as compared with controls.

The present study examines the serum and colon mucosa collected from these same mice and tests them for biomarkers that correlate with the efficacy of the intervention. The research team found that the proinflammatory cytokine interleukin-6 (IL-6) was an indicator of response in both the serum proteins and the gene transcripts of the colon mucosa in mice. Bean-fed mice had significantly lower levels of IL-6 in serum and had changes in many inflammation-associated genes in mucosa. Inflammation plays an important role in colon carcinogenesis, so these changes in inflammation-associated molecules likely play a functional role in modifying the disease process. It was noteworthy that the bean diet counteracted the effect of the carcinogen on colon IL-6.

“Biomarkers of response are important because we would like to match the intervention with those likely to respond. If we can identify after short-term exposures to the intervention those likely to respond, we can save a lot of time and money in human studies,” said Dr. Colburn.

Following the recommendation of the NCI Translational Research Working Group (TRWG) (<http://www.cancer.gov/trwg>) to connect mouse to human trials, Dr. Colburn and colleagues have discovered a mouse-human correlation between a dietary change and a reduction in colon cancer risk. Using an obese mouse model, the team validated the effectiveness of a dietary intervention and then identified a biomarker indicative of healthful changes in mouse colon mucosa. Next the researchers went back to a human clinical trial (the PPT) to validate the biomarkers they identified in their mouse study. The data for this trial, which was presented in April 2009 at the American Association for Cancer Research Annual Meeting, showed that diet may reduce the recurrence of colon polyps in humans by attenuating IL-6, so IL-6 appears to be a predictive biomarker of response to dietary prevention of colon carcinogenesis.

To learn more about Dr. Colburn's research, please visit her CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=colburn>.

Recent CCR Awards

Elected to the National Academy of Sciences

Douglas R. Lowy, M.D.
Laboratory of Cellular Oncology

Life Raft Humanitarian of the Year Award

~ For his work in creating the Pediatric GIST (gastrointestinal stromal tumor) Clinic

Lee Helman, M.D.
Pediatric Oncology Branch
Scientific Director for Clinical Research

Brinker Award for Scientific Distinction

~ For her work in tumor metastasis, identifying the first metastasis suppressor gene, and bringing this research to clinical trial, as well as pioneering work on brain metastasis of breast cancer

Patricia S. Steeg, Ph.D.
Laboratory of Molecular Pharmacology

2009 Alfred Tissieres Young Investigator Award from the Cell Stress Society International

~ For his exceptional work in chaperone biology research

Wanping Xu, M.D., Ph.D.
Urologic Oncology Branch

Norman P. Salzman Memorial Award in Virology

~ For his work on the molecular architecture of native HIV-1 gp120 trimers

Alberto Bartesaghi, Ph.D.
Laboratory of Cell Biology

Norman P. Salzman Memorial Mentor Award in Virology

~ For his work as Alberto Bartesaghi, Ph.D.'s, mentor

Sriram Subramaniam, Ph.D.
Laboratory of Cell Biology

14th Annual AACR Joseph H. Burchenal Memorial Award for Outstanding Achievement in Clinical Research

W. Marston Linehan, Ph.D.
Urologic Oncology Branch

Barcelona Award

~ For technology that made possible the development of the HPV vaccine

Douglas R. Lowy, M.D.
Laboratory of Cellular Oncology

John T. Schiller, Ph.D.
Laboratory of Cellular Oncology

Marsha Rivkin Center for Ovarian Cancer Research Pilot Study Award

~ For her project "Investigation of Genetic Alterations Promoting NF-kappaB in Ovarian Cancer"

Christina M. Annunziata, M.D., Ph.D.
Medical Oncology Branch and Affiliates

Elected to the Board of Governors of the American Academy of Microbiology

Susan Gottesman, Ph.D.
Laboratory of Molecular Biology

American Society of Health-System Pharmacists Research and Education Foundation Award

~ For his sustained contributions to the literature of pharmacy practice

William D. Figg, Sr., Pharm.D.
Medical Oncology Branch and Affiliates

3rd AACR Princess Takamatsu Memorial Lectureship

~ To recognize an individual scientist whose novel and significant work has had or may have far-reaching impact on the direction, diagnosis, treatment, or prevention of cancer

Curtis C. Harris, M.D.
Laboratory of Human Carcinogenesis

Elected to the American Academy of Microbiology

Giorgio Trinchieri, M.D.
Laboratory of Experimental Immunology

Jeffrey N. Strathern, Ph.D.
Gene Regulation and Chromosome Biology Laboratory

Amar J.S. Klar, Ph.D.
Gene Regulation and Chromosome Biology Laboratory

Fellows in the American Association for the Advancement of Science

~ For his seminal contributions to the chemical biology of the bioregulatory molecule nitric oxide

David A. Wink, Jr., Ph.D.
Radiation Biology Branch

~ For his pioneering work on characterizing the cellular and molecular biological aspects of liver stem cells and their role in carcinogenesis

Snorri S. Thorgeirsson, M.D., Ph.D.
Laboratory of Experimental Carcinogenesis

Risky Business:

MYH9 Risk Variants Explain Increased Burden of Kidney Disease among African-Americans

Chronic kidney disease affects more than one in ten individuals in the United States alone, either due to specific kidney disorders such as focal segmental glomerulosclerosis (FSGS) or in association with other illnesses such as diabetes, hypertension, lupus, and HIV. Tracking the genes involved in kidney disease, as with any complex disease, can be challenging—in the case of FSGS, for example, over 10 genes have been previously associated, but their polymorphisms explain only a small portion of the disease burden.

In the October 2008 issue of *Nature Genetics*, Cheryl Winkler, Ph.D., Head of CCR's Molecular Genetics Epidemiology Section, and her colleagues identified—for the first time—variations in a single gene that are strongly associated with kidney diseases. Knowing that these diseases disproportionately affect African-Americans, Winkler and her colleagues relied on admixture mapping to increase the power of their genetic analyses

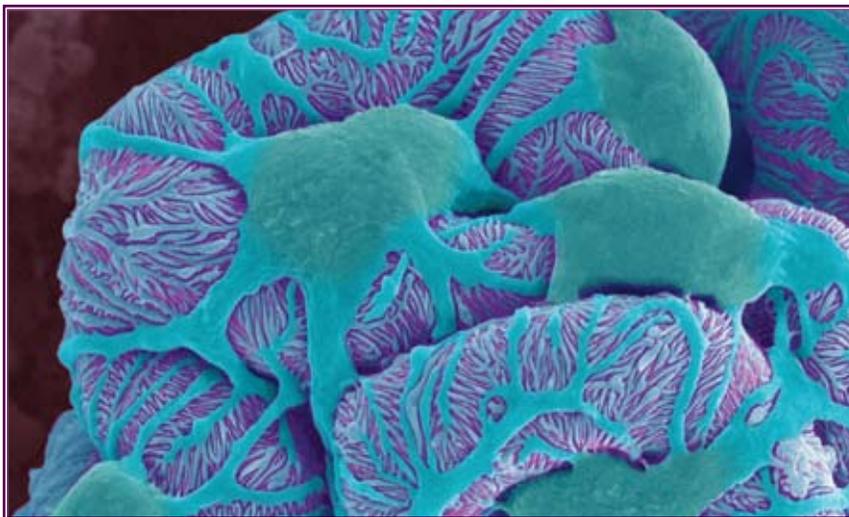
substantially. Based on the hypothesis that risk alleles are present at higher frequency in persons of African descent as compared to European descent, the researchers were able to confine their search to regions of the genome where individuals with the disease have relatively more African ancestry. Using this method followed by fine positional mapping of the candidate gene, they identified several variations in the *MYH9* gene that contribute to

FSGS, HIV-associated nephropathy, and nondiabetic kidney failure.

Their findings reveal that risk among African-Americans with these variants is increased more than four-fold for FSGS, more than six-fold for HIV-associated FSGS (HIVAN), and more than double for nondiabetic kidney failure. About 60 percent of African-Americans carry the risk variants in contrast to less than 3 percent of European-Americans. This large disparity led Dr. Winkler and her team to the conclusion that the increased burden of kidney diseases—especially of FSGS and HIVAN—among African-Americans is substantially due to *MYH9* risk alleles. However, the specific causal variants have not yet been identified.

MYH9-associated kidney disease involves injury to podocytes, cells in the kidney glomeruli (tiny tufts of capillaries that carry blood within the kidneys) that form one of three filtration barriers in the kidney. *MYH9* defects likely produce podocytes that are more susceptible to injury; thus, in the event of a secondary hit (via viral infection, environmental toxins, or other disease), kidney disease develops more easily. In contrast, chronic kidney disease associated with diabetes does not show an association with *MYH9* and may, therefore, be of distinct mechanistic origin.

“This is a finding with excellent bench-to-bed potential—for targeted drug therapy, genetic screening, screening potential donor kidneys, and improved diagnostic decision trees,” said Dr. Winkler. With such a strong risk association, physicians should soon be able to genetically screen patients to identify at-risk individuals and implement preventive measures, including modifiable risk reduction.



(Image: Dennis Kunkel Microscopy, Inc.)

A scanning electron micrograph of a normal podocyte showing the cell body and foot processes surrounding a capillary that forms the kidney glomerulus—the primary blood filtration unit. Kidney diseases associated with *MYH9* are characterized by changes in podocyte structure and glomerular scarring.

To learn more about Dr. Winkler's research, please visit her CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=winkler>.

Keeping Oncogenic HPV in Check: How the Interplay between HPV Oncoproteins and microRNAs Affects Carcinogenesis

For close to a century, researchers have known that certain viruses can cause cancer, but the molecular mechanisms by which this happens are far from understood. A class of molecules discovered relatively recently, microRNAs (miRNAs), appears to play a significant role in cell proliferation and differentiation, and aberrant miRNAs are associated with several cancers. In the April 2009 issue of RNA, Zhi-Ming Zheng, M.D., Ph.D., Investigator in CCR's HIV and AIDS Malignancy Branch, shows for the first time a link between oncogenic viral infection and miRNA expression, which controls cell growth.

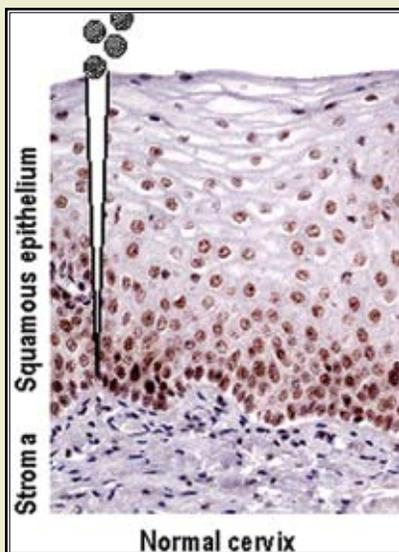
Human papillomavirus (HPV) is a leading cause of genital and anal cancers, accounting for more than 99 percent of cervical cancers and many anal and penile cancers, according to the American Cancer Society. We know that the viral oncoprotein E6 is a critical factor in tumor formation and that it acts to destabilize the tumor suppressor

p53. The p53 tumor suppressor protein, in turn, regulates the transcription of several genes that keep cell proliferation in check by inducing cell cycle arrest, DNA repair, or apoptosis. But which of these myriad targets of p53 are critical for the virus to promote tumor formation?

Tumor-suppressive miR-34a was recently identified as a direct target of the p53 transcription factor. Intrigued, Zheng and his colleagues decided to test the hypothesis that miR-34a might be a critical player in HPV induction of cervical cancer. They found that cervical cancer tissues and cell lines had reduced levels of miR-34a, that viral oncoprotein E6 was necessary for this reduction, and that boosting miR-34a levels in these cells retarded proliferation. "HPV infection controls the cell cycle progression through oncogene E6," said Dr. Zheng. "Previously, we only understood that the oncogene E6 downregulates p53; now we add one more layer to this understanding by finding that miR-34a is regulated by oncoprotein E6. So this is another way to interpret how HPV causes cancer."

Since the publication of this study, Dr. Zheng and colleagues have focused their research on the molecular targets of miR-34a, which remain largely unidentified. In May 2009, the team presented an abstract at the 25th International Papillomavirus Conference and Clinical Workshop in Sweden, revealing a newly identified target of miR-34a: p18, a tumor suppressor and checkpoint component of the cell cycle. Dr. Zheng noted, "By understanding how p18 is targeted by miR-34a, we may be able to use miR-34a and p18 as markers for the diagnosis and prognosis of cancer." He added, "Our study provides the first evidence that viral proteins regulate cellular miRNA expression. So this could be a clue to what proteins for other cancer-causing viruses—not just HPV—do."

To learn more about Dr. Zheng's research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=zheng>.



(Image: Z. Zheng, CCR)

Human papillomavirus infects the cervix at the squamous epithelium.

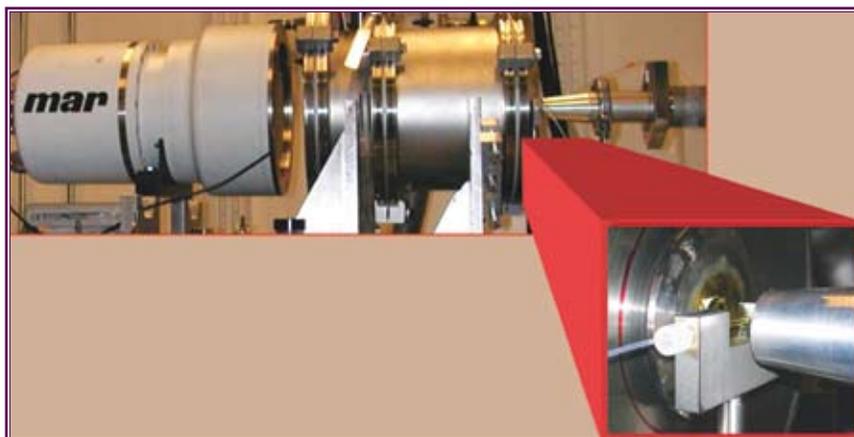
A New PUP for CCR

Determining the proteins, nucleic acids, and the complexes they form in the cell is a daunting task recently made easier for CCR scientists. A new Partnership User Program (PUP) with the Advanced Photon Source of the Argonne National Laboratory gives CCR researchers access to a high-flux, brilliant X-ray beamline used to perform small- and wide-angle X-ray scattering (called SAXS and WAXS, respectively). The data they generate with SAXS/WAXS gives scientists an unprecedented view of complex biological macromolecules in solution.

SAXS complements X-ray crystallography and solution nuclear magnetic resonance (NMR) spectroscopy by providing the full outline of a molecule in solution. It also is used to discover the molecular weight of large proteins in solution, aggregation states of proteins and RNAs, molecular interactions, and protein and RNA folding and unfolding.

Since PUP began, many CCR and extramural laboratories have put the beamline to work using SAXS to:

- Determine protein-protein complexes that play roles in cell migration/adhesion processes, in Jak-Stat signaling, and in the ubiquitination pathway. (Yun-Xing Wang, Ph.D., Head of the Protein-Nucleic Acid Interactions Section of the Structural Biophysics Lab [SBL] at CCR, and R. Andrew Byrd, Ph.D., Chief of the SBL)
- Combine SAXS with NMR to discover the global structures of large RNA molecules in solution. (Yun-Xing Wang, Ph.D.)
- Provide important information on the size and shape of the structural proteins of HIV and other retroviruses. (Alan Rein, Ph.D., Head of NCI's HIV Drug Resistance Program)



(Photo: Argonne National Laboratory)

Advanced Photon Source at the Argonne National Laboratory

- Study the structural biology of the Rev Response Element, a region in the HIV virus that signals for viral RNA to exit the host cell's nucleus. (Stuart Le Grice, Ph.D., Head of the Center of Excellence in HIV/AIDS & Cancer Virology at CCR)
 - Characterize the dimerization state of α -lactalbumin and its mutants in different conditions. (Pradman Qasba, Ph.D., of CCR's Nanobiology Program)
- Beyond CCR, scientists from the National Institute of Diabetes and Digestive and Kidney Diseases and academe—the University of Wisconsin, the University of California at Los Angeles, Johns Hopkins University, the University of Toronto School of Medicine, the Lerner Institute of the Cleveland Clinic, the University of Pittsburgh School of Medicine, and the University of Arizona Pharmacy and Medical School—use the beamline for their research with help from SBL scientists Xiaobing Zuo, Ph.D., and Jinbu Wang, Ph.D.

Young Talent in the Pipeline

Liv Johannsen

CCR intern in the Cancer and Inflammation Program
Mentor: Nadya Tarasova, Ph.D.

- Grand Prize winner at the Frederick County Science and Engineering Fair
- Cash award at the Intel International Science and Engineering Fair
- Full scholarship from the University of Maryland College Park

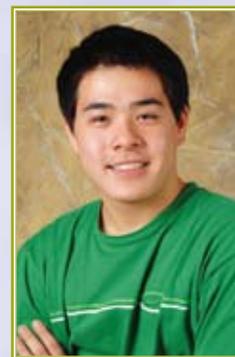


(Photo: SAIC-Frederick Photography)

Marvin Gee

CCR intern in the Laboratory of Comparative Carcinogenesis
Mentor: Yih-Horng Shiao, Ph.D.
Sponsor: Lucy Anderson, Ph.D.

- Semifinalist in the Intel Science Talent Search



(Photo: SAIC-Frederick Photography)

Staff News at CCR

new tenure-track scientists



(Photo: M. Spencer)

C. Ola Landgren, M.D.

Landgren joins CCR's Medical Oncology Branch. He received his M.D. in 1995 from the Karolinska Institutet in Stockholm, Sweden. In 2004 he came to the NCI, Genetic Epidemiology Branch, DCEG, where he worked as a Principal Investigator. His research focuses on treatment-, host-, disease-, and immune-related factors in the pathway from precursor to full-blown hematologic malignancy and their relation to outcome.



(Photo: M. Spencer)

Yinling Hu, Ph.D.

Hu joins CCR's Laboratory of Experimental Immunology, Inflammation and Tumorigenesis Section. She received her undergraduate degree from the Chinese Academy of Medical Science and her Ph.D. from the University of Melbourne in Australia. Hu's research interests are to understand the physiological activities of IKK α in skin tumorigenesis and inflammation and reveal the mechanisms of how IKK α regulates these functions by using genetic animal models.



(Photo: M. Spencer)

Yamini Dalal, Ph.D.

Dalal joins CCR's Laboratory of Receptor Biology and Gene Expression. She received her Ph.D. in 2003 from Purdue University for her work on nucleosome positioning in mammalian cells. She then went on to do postdoctoral research with Steven Henikoff, Ph.D., where she and colleagues discovered unusual properties associated with the centromere-specific chromatin in *Drosophila*. Her research program focuses on the interplay between chromatin ultra-structure and epigenetic regulation.



(Photo: M. Spencer)

Joseph Ziegelbauer, Ph.D.

Ziegelbauer joins CCR's HIV and AIDS Malignancy Branch. He received his Ph.D. from the University of California at Berkeley while in the laboratory of Robert Tjian, Ph.D. He later was a Damon Runyon Cancer Research Fellow with Don Ganem, M.D., at the University of California, San Francisco. He plans to utilize a new method he developed to study the functions of viral microRNAs in the context of cancer biology.

newly tenured CCR scientists

**Raffit Hassan
M.D.**
Laboratory of
Molecular Biology

**Vladimir L. Lariionov
Ph.D.**
Laboratory of
Molecular Pharmacology

**Susan Mackem
M.D., Ph.D.**
Cancer and
Developmental Biology
Laboratory

**Daniel W. McVicar
Ph.D.**
Cancer and Inflammation
Program/Laboratory of
Experimental Immunology

new tenure-track scientists



(Photo: M. Spencer)

Jing Huang, Ph.D.

Huang joins CCR's Laboratory of Cancer Biology and Genetics. He received his B.A. from Peking University and his Ph.D. from the University of Rochester. After finishing his postdoctoral training at the Wistar Institute, he joined the Laboratory of Cancer Biology and Genetics in 2008 to study cancer epigenetics.



(Photo: M. Spencer)

Brian A. Lewis, Ph.D.

Lewis joins CCR's Metabolism Branch. He received his Ph.D. in molecular biology from Princeton University, followed by postdoctoral fellowships at Harvard Medical School and the University of Medicine and Dentistry of New Jersey. He came to the NIH as a Visiting Scientist for two years before joining the Metabolism Branch as an Investigator in October 2008. The lab studies the eukaryotic transcriptional biochemistry of RNA polymerase II, the core promoters, and B-cell promoters.



(Photo: M. Spencer)

Hyun Park, Ph.D.

Park joins CCR's Experimental Immunology Branch. His research focuses on the role and mechanism of cytokine receptor regulation and signaling in immune cells. Park received his Ph.D. from the University of Wurzburg in Germany and completed his postdoctoral training at the Korea Research Institute of Bioscience and Biotechnology and, until most recently, at the NIH CCR.



(Photo: M. Spencer)

Li Yang, Ph.D.

Yang joins CCR's Laboratory of Cancer Biology and Genetics. She received her Ph.D. and postdoctoral training in the Cancer Biology Department at Vanderbilt University. Her laboratory is devoted to mechanisms of inflammation underlying tumor initiation, invasion, and metastasis, with the emphasis on the contribution of TGF-beta signaling and COX-2 pathways.

Going after the Real Killer: Metastatic Cancer

Until recently, metastatic disease was considered part of the continuum of cancer progression resulting from accumulated mutations—a late stage of a unified disease process in which primary tumor cells acquire the ability to migrate away from their initiation site to invade and proliferate in different organs. Although it is true that metastases exert their life-threatening effects well after the primary tumor has become a cause for serious concern, recent research indicates that the seeds of metastatic destruction are sown relatively early on. Furthermore, several lines of evidence suggest that metastatic disease operates through molecular mechanisms distinct from those involved in the development of primary tumors.

Within CCR, several principal investigators are converging on the importance of research specifically aimed at stopping cancer metastases. “The emphasis to date in cancer research and in pharmaceutical development has been on trying to treat and eradicate the primary cancer,” noted Jeffrey Green, M.D., Head of the Transgenic Oncogenesis and Genomics Section in CCR’s Laboratory of Cancer Biology and Genetics. “And the therapeutic strategies for treating primary tumors may not be the same as those needed to treat metastases.”

Kent Hunter, Ph.D., Head of the Metastasis Susceptibility Section, which is also in CCR’s Laboratory of Cancer Biology and Genetics, agrees. “For breast cancer and many other cancers, we all focus on the primary tumor. That’s

the wrong thing to focus on because, more often than not, you solve the primary tumor with surgical resection. What kills people is metastasis.”

Metastasis Suppressor Genes

More than 20 years ago, as a Postdoctoral Fellow new to NCI, Patricia Steeg, Ph.D. (now Head of the Women’s Cancers Section of CCR’s Laboratory for Molecular Pharmacology), launched her quest to study the difference between tumor cells that metastasize and those that do not. She decided to study the differences in gene expression between metastasizing and non-metastasizing cell lines derived from the same tumor, hoping to find genes highly expressed in metastatic lines. It was not until she

heard a seminar describing the first tumor suppressor gene, *Retinoblastoma* (*Rb*), that she realized the significance of a gene she called *Nm23* (non-metastatic gene 23), whose expression was instead reduced in metastatic cell lines. Steeg and her colleagues reintroduced *Nm23* into a highly metastatic melanoma cell line and found that although the cells still made primary tumors when injected into mice, there was a 90 percent reduction in metastases. *Nm23* would be the first identified metastasis suppressor gene.

“Initially, that was an extraordinarily controversial observation,” remembered Steeg ruefully. “People looked at metastasis back then and said it was too heterogeneous and unstable to have consistent molecular pathways underlying it.” There are now, however,



(Photo: R. Baer)

Patricia Steeg, Ph.D.

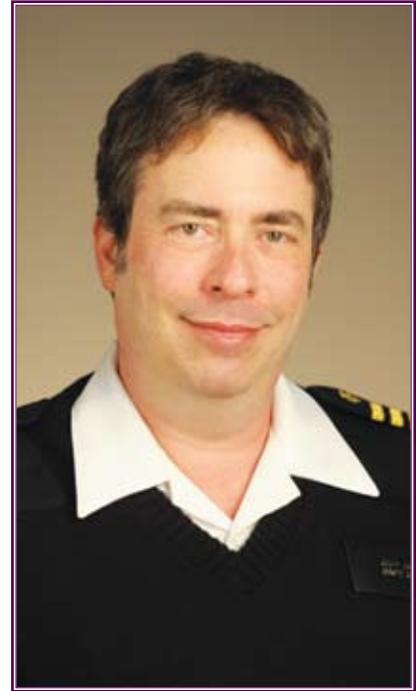
more than 20 known metastasis suppressor genes. These genes are not effective in stopping the growth of primary tumors, but they do stop spreading and/or growth at a distant site. “You have to come to the conclusion that growth of a primary tumor is fundamentally different than the growth of a metastasis.”

And where it has been studied, a number of preclinical drug studies have found differential sensitivity of primary and metastatic growth. “We are trying to treat metastatic disease, but we are not developing drugs for it,” cautioned Steeg even as she attempts to redress this therapeutic imbalance.

A proportion of breast cancers lose expression of the *Nm23* gene. Steeg and her colleagues showed that high-dose medroxyprogesterone acetate (MPA)—a synthetic progestin hormone used historically in the treatment of endometrial cancers as well as a component of hormone replacement therapy—works atypically through a class of steroid receptors (glucocorticoid receptors) not normally associated with progestin to turn expression of the *Nm23* gene back on. The researchers went on to demonstrate in a mouse model of breast cancer metastasis to the lungs that MPA caused a 60 percent reduction in overt

lung metastases by the end of the study. Kathy Miller, M.D., at the University of Indiana University’s Simon Cancer Center is currently leading a Phase II multicenter trial for the use of MPA in the treatment of metastatic breast cancer, a study that stems from Steeg’s preclinical work on *Nm23*.

Steeg and her colleagues are also looking for other targets in the *Nm23* pathway that may influence metastasis. To find molecular targets that are suppressed by *Nm23* and potentially involved in promoting metastasis, they have asked which genes are expressed in a pattern that inversely correlates with *Nm23* expression. One promising candidate, EDG2 (endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2), appears to be sufficient to restore metastatic growth to cells in which *Nm23* functions



(Photo: R. Baer)

Jeffrey Green, M.D.

“You have to come to the conclusion that growth of a primary tumor is fundamentally different than the growth of a metastasis.”

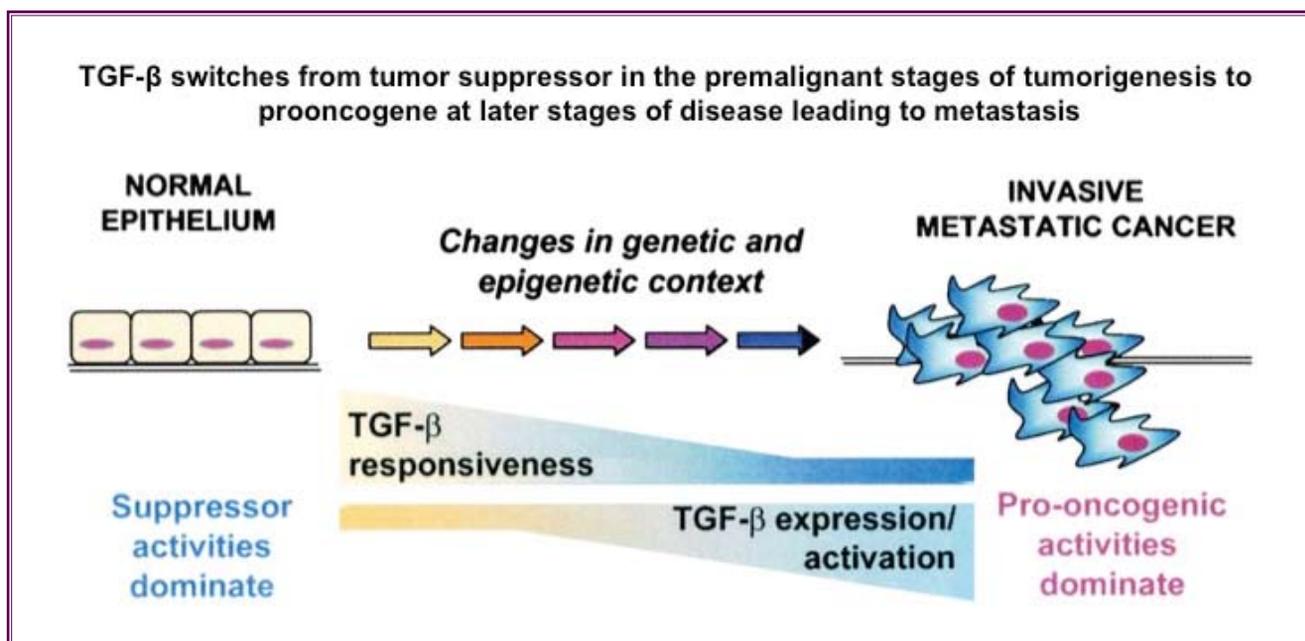
as a metastasis suppressor. Steeg’s team is currently asking whether EDG2 inhibitors will have anti-metastatic effects in preclinical models.

One Molecule, Two Different Effects on Cancer

Around the same time that Patricia Steeg was embarking on her work with metastasis suppressor genes in the 1980s, Lalage Wakefield, D.Phil., now Head of the Cancer Biology of TGF- β Section in

CCR’s Laboratory of Cancer Biology and Genetics, was also beginning her work as a Postdoctoral Fellow at NCI on another molecular player in metastasis. However, it took her a little while to realize where her research was leading.

“TGFs [transforming growth factors] had just been described in the literature,” explained Wakefield, an echo of the excitement from those early days still in her voice. TGFs were secreted by cancer cells and were able to transform normal fibroblasts into a premalignant state. “It



TGF- β switches its role from suppressing tumors before malignancy sets in to promoting metastasis at later stages of disease.

seemed to me that TGFs were going to be the answer to cancer. If we could purify and block these factors, then that would be cancer cured."

TGF- β was eventually discovered to have multiple roles in several different tissues and cell types. It was found to be a key regulator of immune system function, as well as a potent inhibitor of proliferation of normal epithelial cells. Importantly, the TGF- β pathway was genetically inactivated in a number of different cancer types and became known, paradoxically, as a tumor suppressor. Several preclinical studies and mouse models later, the dual role of TGF- β in cancer progression was finally revealed. In the early stages of cancer progression, TGF- β does indeed have tumor suppressor activity, inhibiting proliferation and maintaining genomic stability. As cancer progresses, tumor cells progressively alter their responsiveness to TGF- β . At that stage, TGF- β promotes cell migration, promotes invasion of cancer cells into different tissues, and becomes a pro-survival factor. Meanwhile, TGF- β acts on other cell types, such as fibroblasts, to promote angiogenesis, secrete different

types of molecules into the extracellular matrix, and suppress immune surveillance. In short, TGF- β can promote metastasis through multiple routes.

"TGF- β is a master regulator that sits at the interface of the tumor [and its cellular environment]. It affects every cell that comprises that ecosystem," concluded Wakefield. A molecule with so many diverse effects, operating differently at different stages of cancer progression, would seem to be a pharmaceutical drug developer's nightmare. No one was more surprised than Wakefield and her colleagues, therefore, when they were able to genetically engineer a mouse to encode an inhibitor of TGF- β in its genome and found that this inhibitor protected mice against metastasis in a genetic model of breast cancer. The team has since followed up this work with further preclinical studies that support the use of TGF- β inhibitors to treat metastatic cancer in the clinic. As a result, NCI investigator John Morris, M.D., is now leading a Phase I clinical trial to test GC1008, a human monoclonal antibody against TGF- β in patients with locally advanced or metastatic renal

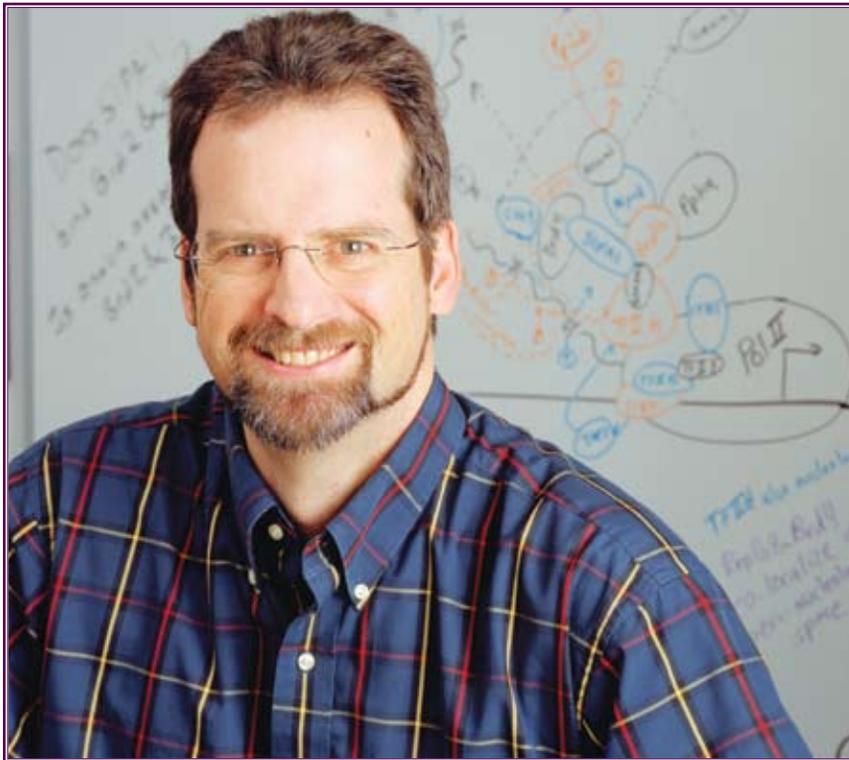
cell carcinoma or malignant melanoma. The trial is in an extension phase at the highest dose and appears to be showing some promising effects.

"It's been an incredibly exciting story so far because I have seen this molecule go from its initial discovery and identification to clinical testing, and believe me, it was not a straightforward process," said Wakefield.

Genetic Susceptibility

No one doubts that acquired mutations in individual genes play a critical role in cancer. But, noted Hunter, "You can't look at these things in isolation." He cites the fact that women with *BRCA1* mutations do not always develop cancer. "You have to understand the genetic context."

Hunter has taken a population genetics approach to ask whether there are inherited risk factors associated with metastatic progression in cancer. Using a transgene to induce metastatic mammary tumors in several genetically distinct strains of mice, Hunter has shown that the metastatic efficiency, as measured by the density of pulmonary metastases in these mice, varies enormously with



(Photo: R. Beier)

Kent Hunter, Ph.D., looks for inherited risk factors associated with metastatic progression.

genetic background. Polymorphisms—DNA sequence differences among individuals—account for variations in many normal physiological traits, such as body size and coloring. Polymorphisms also account for different levels of gene expression, and they account for variation in primary tumors from a variety of tissues. In hindsight, then, it is not surprising that inherited genetic differences would affect the development of metastatic cancer.

“We’re taught to reduce complexity... But we actually have to embrace it.”

In humans, research has shown that metastatic cells, like primary tumor cells, can be characterized by a gene expression

“signature” and that this signature can be used to predict the likelihood of metastasis. Although Hunter does not dispute these findings, and has even found the same genetic signatures in mice with high risk of developing metastasis, he does argue against the interpretation that this signature represents only the accumulation of genetic mutation creating metastatic cells. Instead, he has shown that these signatures can be explained by an interaction of both mutation and genetic background and that non-cancerous tissue from animals with high metastatic risk also has similar gene expression profiles.

But, despite the growing body of evidence that he and his colleagues have developed, skeptics remain. Hunter thinks part of the difficulty is in the scientific culture. “We are trained to think in terms of somatic mutation as cancer biologists. And there’s a big divide between susceptibility and somatic genetics in which defects acquired from genetic mutation and rearrangement—not inheritance—are at play.

“We’re taught to reduce complexity,” concluded Hunter. “But we actually have to embrace it.”

The Extracellular Matrix

Hunter and his team have been working to identify the genes that underlie risk of metastatic disease. One focus of their work is the extracellular matrix (ECM), the complex molecular environment that cells both secrete and live in, which provides physical scaffolding through which cells migrate as well as transmit signals to and from cells. Many studies, including Hunter’s own work, have shown that changes in the expression of genes encoding ECM molecules predict metastatic progression in both human breast cancer and mouse models. Hunter and his colleagues have begun to identify factors that specifically modulate both ECM-related gene expression as well as metastatic tumor progression. Although we are still far from a mechanistic understanding of how changes in ECM gene expression impact metastasis, the relationship makes some intuitive sense. “I think it has to do with the way cells sense their microenvironment through ECM signaling,” said Hunter. For example, the ECM could sequester or modulate the availability of TGF- β and other cytokines involved in growth and immune regulation.

Jeffrey Green and his colleagues have also followed up on the evidence for involvement of the ECM in metastasis. Like Hunter, Green has wondered whether it is not the accumulation of new genetic abnormalities that causes a disseminated but dormant tumor cell to proliferate into clinical disease, “but that something else in the immediate environment or within the host may lead to the trigger that allows these cells to proliferate.” Green suspects that there may be critical changes in the composition and structure of the ECM that could allow tumor cells to read different stimulatory signals and initiate a proliferative response.

But dormancy really just means that the disease is subclinical and that doctors cannot see it. How do you find a dormant cell to study it? “Dormancy,” Hunter explained, “gives people the idea that it’s an inactive seed, a spore sitting somewhere. That’s obviously not true—they are cells. We don’t know if they are static, or patrolling the body like a lymphocyte.”

Green and Dalit Barkan, Ph.D., a Visiting Scientist, recently reported the development of a three-dimensional culture system model of metastatic cancer that will allow them to address some of the questions of molecular and cellular mechanisms that are so difficult to tackle in this disease. They have shown that cell lines that proliferate in normal cell culture but that can be distinguished by their metastatic potential *in vivo* can also be distinguished in their three-dimensional culture system. Thus, they have been able to study the transition from quiescence to proliferation of metastatic cells, and they have demonstrated a role for the extracellular microenvironment in regulating the reorganization of internal cellular structure that occurs during the switch from dormancy to proliferation. The molecules involved in this reorganization could represent additional targets for metastatic inhibitors (see “Let Sleeping Micrometastases Lie” in Vol.2, No.2 of *CCR connections*).

Finding a Cure

“I am not certain that we will ever be able to completely cure metastatic cancer,” said Hunter, realistically and without pessimism. “We should also think about treating it the same way people treat heart disease, by looking for ways to reduce the risk of developing metastatic disease.” Hunter’s lab has shown that high doses of caffeine suppress metastasis in their mouse model. Although the work does

Ultimately, we may need to rethink how we do clinical trials.

not support a recommendation for cancer patients to drink liters of coffee every day, it does indicate that small-molecule agents might be developed for chronic administration to patients that would reduce the risk of metastasis, a strategy



(Image: R. Baer)

Postdoctoral Fellow Daniel Fitzgerald, Ph.D., slices brain tissue for analysis.

analogous to the administration of statins to reduce the risk of heart disease.

Wakefield’s work with TGF- β , which has effects on so many different physiological systems, has led her to the conclusion that combinations of drugs with different molecular targets will be an important part of the solution. Her work suggests that a combination of a lot of small effects on different cell types involved in the metastatic process would be most effective in combating the disease.

“The major stumbling block,” Steeg pointed out, “is how to test our preclinical data in the clinic. Most of our data says that if we use drug X, we can prevent metastasis, but standard clinical trials start with a Phase I trial in highly metastatic patients—so you are asking a drug to melt a golf ball-sized tumor. Most agents will fail in that trial design [even though they might be effective

when administered earlier].” Steeg suggests that including biopsies that demonstrate whether the drug had an effect on its target may be a first step. Better imaging tools will also be critical. But, ultimately, we may need to rethink how we do clinical trials.

Steeg has recently formed a Center of Excellence to study brain metastases of breast cancer, a disease that combines all of the difficulties in studying metastatic disease with the need to find drugs that cross the blood-brain barrier that normally protects the brain from most blood-borne molecules. The current standard of care, whole brain radiation therapy, may be successful in eradicating the tumors for a time, but it may have serious neurological side effects. The Center’s work, which has been funded by a five-year grant of over \$17,000,000 from the Department of Defense Breast Cancer Research



(Photo: R. Baer)

Lalage Wakefield, D.Phil.

Program, is a comprehensive program ranging from target identification to drug delivery methods. The researchers that form this center include neuropathologists, neurosurgeons, neuro-oncologists, molecular biologists specializing in breast cancer, and experts on the blood-brain barrier. "We each have our assignment—we need more model systems, and we need more tissue studies," Steeg concluded (see also "Small Molecule, Big Impact" in Vol.2, No.2 of *CCR connections*).

To learn more about Dr. Hunter's research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=hunter>.

To learn more about Dr. Green's research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=green>.

To learn more about Dr. Steeg's research, please visit her CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=steeg>.

To learn more about Dr. Wakefield's research, please visit her CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=wakefield>.

The Virtual Metastasis Research Lab



(Image: Epstein/Kean Healthcare)

The Virtual Metastasis Research Lab (VMRL) comprises laboratories from several cities across North America.

Virtual Metastasis Research Lab

Every month, a group of cancer researchers gets together to discuss the latest results of their work in an informal setting. They discuss unpublished results, solicit each other's help in understanding their data, and toss around a few wild ideas. This situation sounds like a typical lab meeting, except that the researchers come from many different laboratories, both within CCR and at universities across the country, and they meet online using Web-based conferencing tools.

Kent Hunter, Ph.D., organized the Virtual Metastasis Research Lab (VMRL), which evolved from a CCR working group on metastasis. "Metastasis is an organismal disease," said Hunter, noting that solving it will require researchers with a diverse set of expertise. "Lots of different views on the same data open up interesting ideas for people to try. Everyone contributes in different ways."

Lalage Wakefield, D.Phil., agrees. "It's very interactive, there's a lot of discussion, and we really benefit from

having great people on the outside as well as the ones we have here." Wakefield also likes the way it has helped to encourage collaboration within CCR. She points to the development of a lung slice culture system to study the early events of metastatic cell seeding that started as a casual conversation between her and Hunter about the need for an intermediate system between purely *in vitro* approaches and animal models. They took their notion to their colleague Chand Khanna, D.V.M., Ph.D., Head of the Tumor and Metastasis Biology Section in CCR's Pediatric Oncology Branch, who turned around and created it.

The VMRL has entered its third year, and it includes approximately 50 people from the participating laboratories. "I think it's helped bring people within NCI as well as the extramural participants much closer together," said Jeffrey Green, M.D. "Instead of seeing them at a meeting once a year, we talk to each other all the time."

Cancer Research Takes Flight:

Wnt Signaling in Development and Disease

What do cancers have in common with fruit fly wings? Wnts. The very name of the Wnt (pronounced /wint/) family of secreted signaling molecules proclaims its dual history in developmental biology and cancer research. The “w” comes from wingless, a gene necessary for the proper development of the fruit fly body plan. The “nt” comes from Int oncogenes, first identified near sites of integration of the mouse mammary tumor virus. There are 19 Wnt genes in the human genome. Their tight regulation orchestrates development both embryonically and into adulthood; their misregulation contributes to multiple cancers. The merging of these two lines of research, which is now more than 25 years in the making, has been a boon for both fields.

Terry Yamaguchi, Ph.D., Head of the Cell Signaling in Vertebrate Development Section in CCR’s Cancer and Developmental Biology Laboratory, came to Wnt signaling from developmental biology. His research has taken him from an interest in the late stages of muscle differentiation steadily backwards to the role of Wnts in the earliest steps of cell specification from embryonic stem cells. Now he hopes to define the key molecular events that govern the fate of stem cells in embryonic development and to apply that knowledge to understanding how stem cells contribute to adult tissues normally as well as how abnormal signaling gives rise to cancers.

Location, Location, Location

The mantra of “location, location, location” is as critical for determining cell fate during development as it is for setting the value of real estate, but location works its magic in development through the much more complicated process of gene regulation. Embryonic

stem cells are originally pluripotent—capable of developing into almost any cell type, but their fates are gradually refined as they interact with their local environment. The pluripotent embryonic stem cells soon give rise to three germ layers—ectoderm, mesoderm,

and endoderm—which give rise to specific tissues. Gradients of secreted signaling molecules activate distinct gene expression programs in the cells of each germ layer, which in turn regulate the cells’ interaction with the gradients of signaling molecules they encounter.



(Photo: R. Baer)

Terry Yamaguchi, Ph.D. (*right*), with Postdoctoral Fellow Bill Dunty, Ph.D. (*left*), and technician Kirstin Biris (*center*).

The trick throughout development is to create signals that are sufficiently restricted in time and space that they balance the production of more stem cells (proliferation) with the production of specific cell types (differentiation).

The trick throughout development is to create signals that are sufficiently restricted in time and space that they balance the production of more stem cells (proliferation) with the production of specific cell types (differentiation) to produce exactly and only as many cells as necessary for a specific tissue, a concept known as stem cell homeostasis.

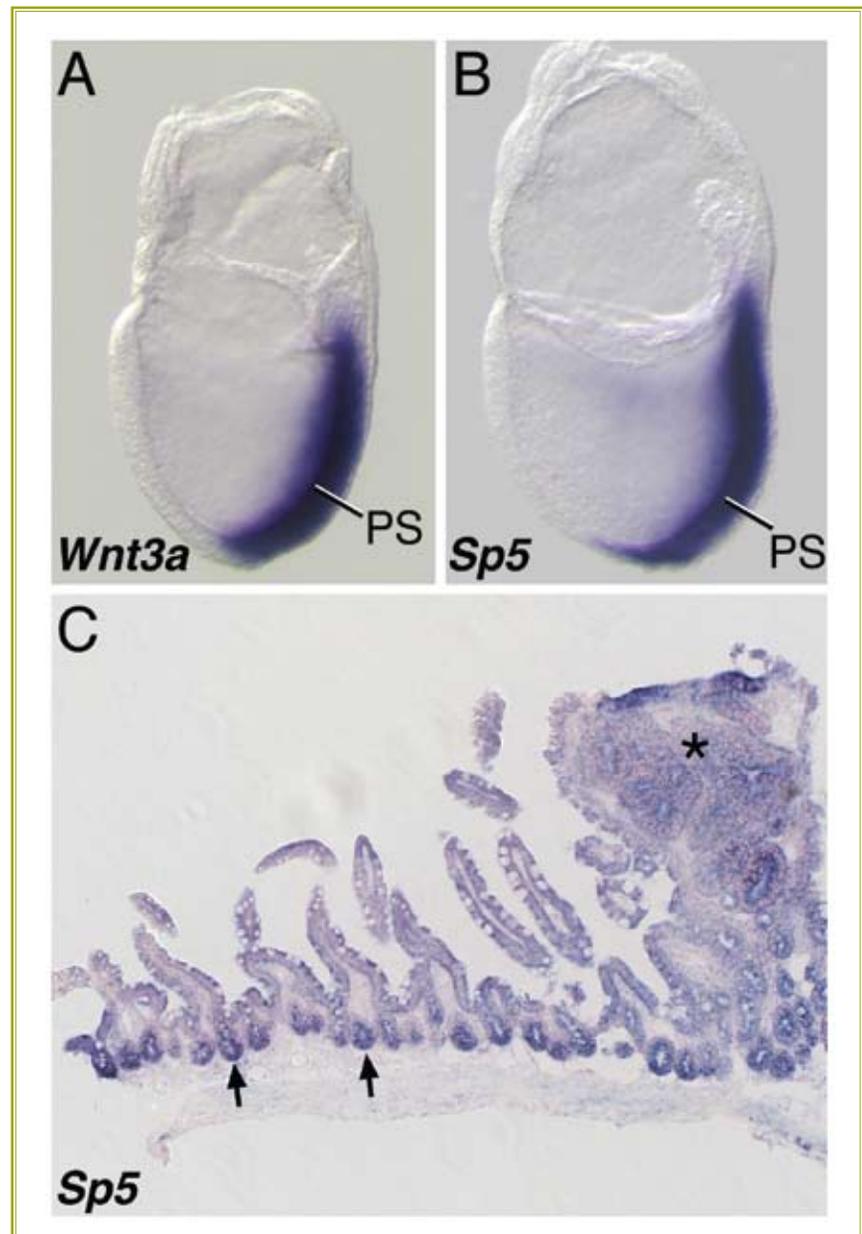
The 19 Wnt ligands are generally expressed in patterns that are tightly regulated in time and space throughout development. Mutation of these genes usually results in dramatic developmental defects, although there appears to be some redundancy in the system so that a single *Wnt* mutation may leave an embryo seemingly unimpaired. Without *Wnt3a*, for example, the entire trunk and tail mesoderm fails to form. To similarly “disappear” the lungs, however, requires the double mutation of *Wnt2* and *Wnt2b*.

“The primitive streak,” described Yamaguchi, pointing to a dark purple line in a micrograph of an eight-day old embryo, “is a source of many secreted signaling molecules, including *Wnt3a*, which can pattern the entire anteroposterior axis.” Whereas it was once believed that the primitive streak was simply a point through which cells transit as they become the mesoderm of the trunk and tail, it now seems that the primitive streak is also a source of stem cells that give rise to the germ layers. “One of the main hypotheses that we are pursuing is that *Wnt3a* in the primitive streak is required for the maintenance of mesodermal stem cells.”

Through a series of genetic experiments, published in the January 2008 issue of *Development*, Yamaguchi, Postdoctoral Fellow Bill Dunty, Ph.D., and their colleagues have formally demonstrated that *Wnt3a* works through the well-studied canonical β -catenin pathway to support mesodermal stem cells. β -catenin is normally maintained at low levels in the cellular milieu by the APC/axin complex, which steadily consigns

β -catenin to degradation. Wnt signaling sequesters some of the components of the degradation complex, resulting in increased levels of β -catenin, which can then make its way to the nucleus to activate the transcription of a number of target genes.

“The main point of this pathway from our perspective is that its stimulation activates a transcriptional program of gene expression. One of the big goals in the lab is to identify what this pathway is doing in the early embryo and identify the target genes through a transcriptional profiling approach.” By looking at the gene expression patterns in different *Wnt3a* and β -catenin mutant mice, Yamaguchi’s team has identified 62 genes that may be regulated by this pathway. Some, like *Sp5* and *Axin2*, are known targets of the Wnt/ β -catenin system in other contexts, some are known oncogenes like *Myc*, and others



(Image: T. Yamaguchi, CCR)

Sp5 is an example of a *Wnt3a*/ β -catenin target gene that is expressed in primitive streak (PS) stem cells of the mouse embryo (panels A and B) and in adult intestinal crypt stem cells (panel C, arrows) and adenomas (asterisk).

appear to be completely novel. Kristin Biris, a technician in Yamaguchi's group, is using *in situ* hybridization to determine where these Wnt3a target genes are expressed in early embryos.

One of the most interesting target genes they have studied so far is *Mesogenin 1*, which is itself not only directly activated by Wnt3a signaling but also appears to operate in a feedback loop to inhibit Wnt3a signaling. Such an inhibitory feedback mechanism could allow high concentrations of Wnt3a, as found in the primitive streak, to support mesodermal stem cell renewal, whereas the effects of lower concentrations of Wnt3a would be inhibited by *Mesogenin 1* feedback, turning a gradient of Wnt3a into a threshold that supports either proliferation or the differentiation of mesodermal stem cells.

Into the Crypt

High concentrations of Wnts are not confined to embryonic development. They reappear, among other places, in the adult intestine, where they regulate the intestinal stem cell niche. "Wnts are so conserved, and their expression is so closely associated with stem cell populations, we believe that what we learn from the early embryo may be generally applicable to other stem cells in the adult," said Yamaguchi. He and his colleagues are now beginning to put that belief to the test.

The adult intestine is coated with a single layer of epithelial cells that are responsible for digestion and absorption, as well as for providing a barrier against pathogens. These epithelial cells, of which there are four major types, are replaced every 4–5 days by a process of cellular renewal. The stem cells that give rise to these new cells are found in pockets of cells called the intestinal crypt. Deep in the crypt, new cells are born and mostly migrate upwards and away from the source of their renewal, up into finger-like protrusions of the intestine called villi. Three days after their cellular identity or fate is sealed, they reach the tip of the villus, self-destruct, and are shed away to be replaced by younger cells.

It turns out that Wnt signaling controls this process of self-renewal,

"The connection of the Wnt pathway to human cancer is very strong—mutations in this pathway are associated with 85–90 percent of human colorectal cancers."

operating as a kind of master switch between proliferation of stem cells and differentiation into epithelial cells. Several studies have shown that Wnt-target gene expression occurs in a gradient that is strongest at the base of the intestinal crypt and weakens further away. And loss of β -catenin, the key transducer of Wnt signaling, dramatically reduces intestinal cell proliferation.

Yamaguchi and his colleagues have compared the gene expression patterns they observed in the embryonic mesoderm with that of the adult intestinal crypt and found a remarkable 60 percent of the Wnt-target genes that they identified in mesodermal stem cells are also

found in the adult intestinal stem cells. Identifying these genes is the first step in establishing the critical molecular network that is responsible for stem cell maintenance, whether in the embryo or in the adult. Functional follow-up studies will be necessary to establish their roles in cellular renewal (see "The Power of Embryonic Stem Cells").

Development Gone Awry

The precise regulation of development, once tampered with, can quickly give rise to abnormal growth that is the hallmark of cancer. "From my developmental perspective, cancer is developmental signaling gone awry," explained



Terry Yamaguchi, Ph.D. (left), with Postdoctoral Fellow Ravi Chalamalasetty, Ph.D. (right).

(Photo: R. Baxter)

Yamaguchi. Already in 1989, mutations of the gene *Adenomatous polyposis coli (APC)* were found in patients with familial adenomatous polyposis (FAP) and in sporadic colorectal cancers before it was understood that *APC* was a critical component of the Wnt signaling pathway. Since then, several other mutations in the canonical Wnt signaling cascade have been associated with cancers.

Hans Clevers, M.D., Ph.D., and colleagues at the Utrecht University Medical Centre, The Netherlands, showed in a paper published in *Nature* this year that deleting *APC* in long-lived intestinal crypt stem cells—but not in differentiated cells migrating away from the intestinal crypt—leads to intestinal adenomas. The transformed stem cells appear to remain in the crypts, steadily fueling growth of the adenomas, and they may represent one of the best examples of a true cancer stem cell.

Studying the same Wnt3a-target genes that he originally identified in embryonic mesoderm, Yamaguchi has shown that 40 percent of these genes are expressed in intestinal adenomas. “So, we’re asking whether any of these genes are required downstream of Wnt signaling for tumor formation.” Specifically, in a mouse model of intestinal adenomas in which the β -catenin pathways are constitutively active, Yamaguchi and his colleagues are asking whether they can reduce the tumor burden by knocking out some of the Wnt-target genes. Conversely, they are also trying to make transgenic mice that overexpress individual target genes specifically in the intestinal epithelium to ask whether they alone are sufficient to form tumors.

“The connection of the Wnt pathway to human cancer is very strong—mutations in this pathway are associated with 85–90 percent of human colorectal cancers. And this case is one where the animal model, although not perfect, is quite good for human cancer. The molecular mutations in both cases are essentially the same. Thus, we have a great opportunity to apply what we know from normal biology to an animal model of cancer.”

For more information about Dr. Yamaguchi's research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=yamaguchi>.

The Power of Embryonic Stem Cells

Embryonic stem cells are not only part of the biology fueling Terry Yamaguchi, Ph.D.'s, intellectual curiosity, but they are also a means to satisfy that curiosity. As a graduate student, Yamaguchi first exploited the ability of embryonic stem cells to differentiate *in vitro* to identify the growth factors important for the specification of mesodermal fates. His work led to the discovery of a family of receptors that regulates the stem cells that give rise to blood and blood vessels. Now Yamaguchi is an enthusiastic adopter of a powerful embryonic stem cell system generated by Michael Kyba, Ph.D.'s, group at the University of Minnesota.

While *in vivo* studies are critical to developmental biology, testing biochemical mechanisms is difficult to do in an embryo of only a few thousand cells. “We wanted to move away from an embryo to a stem cell population we could manipulate *in vitro*,” explained Yamaguchi. Dr. Kyba had recently developed an inducible system for expressing genes of interest in embryonic stem cells in a consistent and reliable way. The gene is always integrated at the same genetic locus, and it is induced by the application of doxycycline.

“These [embryonic stem] cells are a great compromise—we can do so much manipulation genetically and biochemically *in vitro*—and the sky is the limit because the efficiency of targeting is so good,” enthused Yamaguchi. In their laboratory, and with these cells, the probability of a successfully engineered cell is almost 95 percent for any gene of interest. They are, therefore, able to screen all of the Wnt3a target genes they identify by transcriptional profiling for functional activity and biochemical interactions. Postdoctoral Fellow Ravi Chalamalasetty, Ph.D., is using this approach to identify the targets of *Mesogenin 1* (see main text).

Once stable embryonic stem cell lines are engineered with the inducible gene of interest, these cells can be taken back to the embryo to produce a transgenic mouse. Additionally, once the culture conditions are solved, these cell lines can be differentiated *in vitro* into any cell type (e.g., a heart cell or a neuron or, maybe one day, an intestinal stem cell). “For me,” said Yamaguchi, “the possibilities are endless with these embryonic stem cells.”

Balancing Silence:

How a Cell's Fate Is Determined

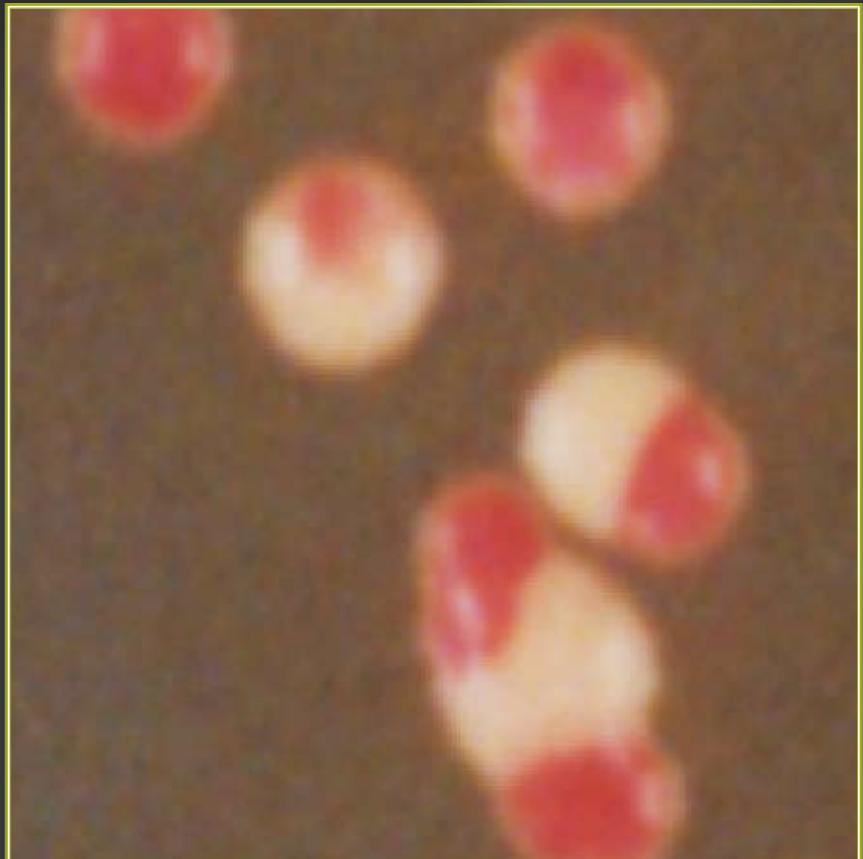
The fate of a cell is determined by more than the string of A's, T's, G's, and C's that make up its DNA sequence.

*Epigenetic regulation of gene expression—governed by the way in which DNA and proteins are packed and interact in the tiny nucleus of a cell—is the reason why two cells with identical DNA can have very different characteristics. For example, one might be a neuron producing electrical signals and the other a pancreatic islet cell producing insulin. Shiv Grewal, Ph.D., Head of the Chromosome Biology section in CCR's Laboratory of Biochemistry and Molecular Biology, has studied this question of phenotypic determination in the fission yeast, *Schizosaccharomyces pombe*, for more than a decade. The mechanisms that he and his team have uncovered appear fundamental to maintaining genomic stability and function, from yeast to man.*

The Mating Habits of Yeast

Among the model organisms biologists use to study genetic mechanisms, the fission yeast *Schizosaccharomyces pombe* has several advantages. A single-celled eukaryotic organism with a small genome that divides rapidly and is easily grown in a laboratory Petri dish, *pombe* nonetheless turns out to share many conserved cellular mechanisms with higher eukaryotes, including man.

For such simple creatures, fission yeast have a complicated sex life that is defined by a single location on their genome—the mating type locus (*mat*). *Mat* encodes three genes, but two of these genes are silenced, and only one is actually expressed in a single organism, thereby defining it as being either an M- or P- mating cell type. Feeding happily in a nutritionally rich environment, fission yeast do not reveal their mating type; it is only when starved that they define their orientation and partner with a cell of the other type to reproduce.



(Image: S. Grewal, CCR)

Fission yeast colonies composed of cells with identical genomes. Red colonies indicate silencing of a gene caused by the spreading of heterochromatin complexes.

Fission yeast can also reproduce asexually, so that a single cell divides to form a colony of clones. What has fascinated cell biologists for decades, however, is the fact that a single clone can give rise to both mating cell types. What genetic mechanisms could account for the mating type switch of just one of two daughter cells? Although this might seem like a somewhat esoteric question in yeast biology, it addresses the most basic notion of how two cells with identical genomes can, in fact, be different. In *pombe*, this vast question comes down to the more experimentally tractable one of how gene silencing at the *mat* locus is controlled—a question Grewal first set out to answer as a Postdoctoral Fellow in the laboratory of Amar Klar, Ph.D. (now Head of the Developmental Genetics Section in CCR's Gene Regulation and Chromosome Biology Laboratory), and has continued working on to this day.

...it addresses the most basic notion of how two cells with identical genomes can, in fact, be different.

Good Chromatin and the Rest

Packaging a double-stranded DNA helix measuring a meter or more in length into the nucleus of a cell measuring less than a millimeter is no small feat of molecular engineering. The genome is highly compacted, with DNA wound around protein complexes called histones to form repeating structures called nucleosomes, which are themselves folded into even more complex structures that eventually form what microscopists see as chromatin material (chromosomes) in the nucleus. Modifying the histone protein complexes by enzymes is an important means of regulating gene expression (see "Histone Modification and Cancer").

Long before Watson and Crick deduced the double helical structure



(Photo: R. Baer)

Shiv Grewal, Ph.D., examines colonies of fission yeast.

of DNA, cell biologists described the genetic material within the cell nucleus as occurring in two forms—heterochromatin and euchromatin—on the basis of their staining patterns under a light microscope. Heterochromatin is a highly condensed form of chromatin in which gene expression is largely silenced, whereas euchromatin is much more loosely configured and enriched with expressed genes. Heterochromatin typically contains DNA with long repeating elements that do not encode genes. Instead, it comprises structurally distinct and important regions of the chromosome such as telomeres (the ends of the chromosomes that need to be protected from enzymes) and centromeres (where the two halves of a chromosome are joined in the middle and where the machinery that segregates chromosomes during cell division attaches).

"For a long time, heterochromatin was looked upon as part of the genome that is silenced as inert static structures," recalled Grewal. "But we know now they are highly dynamic structures that change in response to the cell cycle, developmental and environmental conditions, and stresses. Heterochromatin plays a very important role in a number of cellular processes, including developmental choices." Grewal has been an integral part in creating this new view of heterochromatin.

The Role of RNAi

It turns out that gene silencing at the mating-type locus and centromeres in fission yeast is controlled through

heterochromatin. Early on in his work on mating type switching, Grewal set out to sequence the whole region. "While I was sequencing, I found a repeat element in the middle and thought I had accidentally cloned centromeric repeats," he recalled. Instead, he had discovered that the mating type region also contained these repeats and, furthermore, that knocking out these repeats abolished silencing across the region.

After his postdoctoral fellowship, Grewal started his own laboratory in Cold Spring Harbor in 1998. Searching for new genetic mutations that would alter mating type switching in fission yeast to provide new insights into the mechanism, his laboratory had knocked out the gene *ago* (*argonaute*) because it had recently been shown to affect the similar phenomenon of asymmetric cell division in germ cells. "For six months, we had no phenotype," reported Grewal. "But then I noticed that the cells with *ago* had chromosome segregation problems. These were easy for me to recognize because other factors I worked with as a postdoc, which affected heterochromatic silencing at centromeres, also had chromosome segregation problems." Therefore, he reasoned, *ago* may also be involved in the regulation of heterochromatin.

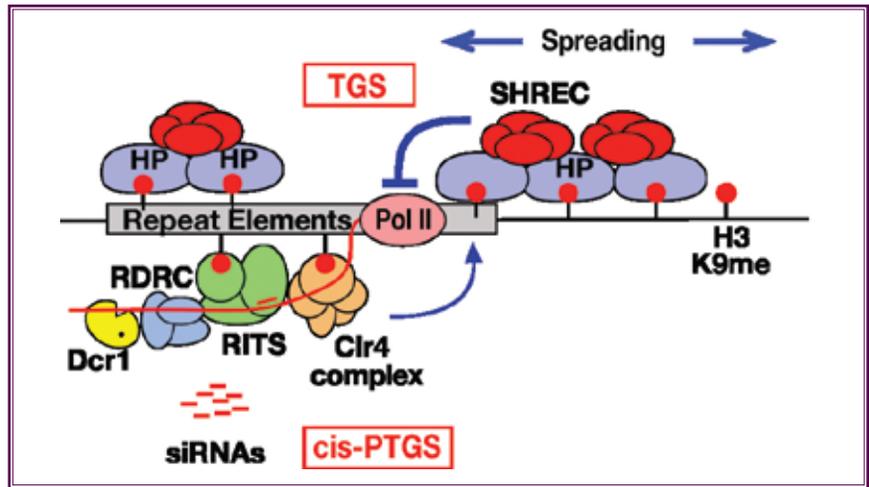
"A lot of things come together in science sometimes," remarked Grewal. Around this time, Craig Mello, Ph.D., published his finding that in worms argonaute was part of the newly described process of RNA interference (RNAi).

RNAi acts by the production of very small pieces of RNA from double-stranded RNA generated at specialized repeating DNA elements. RNAi acts through multiple pathways to regulate gene transcription and translation. (Mello shared the Nobel Prize with his colleague Andrew Fire, Ph.D., for the discovery of this process in 2006.) Meanwhile, Grewal and his Postdoctoral Fellows Junichi Nakayama, Ph.D., and Ken-ichi Noma, Ph.D., were getting ready to publish two papers in *Science* demonstrating that a specific methylation of the histones (designated H3K9) was important for gene silencing at the *mat* locus through the recruitment of so-called chromodomain proteins. The next step was to show that RNAi was necessary both for H3K9 methylation and for gene silencing. Over the next few years, working with his student Ira Hall and Postdoctoral Fellow Ken-ichi Noma, Grewal did just that.

“The key thing from all these studies...is a self-reinforcing loop,” explained Grewal. H3K9 methylation in heterochromatin provides a landing pad for RNAi machinery, which can in turn act on the repeating DNA sequences in the heterochromatin to further recruit silencing machinery. Silencing can also spread from the region of initiation across long stretches of DNA. In fact, studies by Postdoctoral Fellows Takatomi Yamada, Ph.D., Tomoyasu Sugiyama, Ph.D., Songtao Jia, Ph.D., and Tamas Fischer, Ph.D., have led to an important realization that heterochromatin serves as a versatile recruiting platform for factors involved in many cellular processes, including for proteins involved in cell-type switching and proper segregation of chromosomes during cell division.

Blurring the Distinction

“Heterochromatin is not a static, inert structure,” repeated Grewal. “The [chromodomain] proteins not only recruit silencing proteins but also recruit destabilizers to promote transcription... it’s a balance that determines the state.” In particular, work performed by Postdoctoral Fellow Martin Zofall, Ph.D., showed that a single chromodomain protein (called Swi6) recruits not only silencing factors but also anti-silencing factors that facilitate transcription of heterochromatic repeats. Moreover, a paper published in



(Image: S. Grewal, CCR)

Formation of heterochromatin in fission yeast involves coordinated functions of multiple proteins. A self-reinforcing loop couples the generation of short interfering RNAs (siRNAs, indicated as red line segments) from DNA repeat elements with the methylation of histones (H3K9me) via the Clr4 complex. The chromodomain proteins (HP), which bind to methylated histones, recruit gene silencing factors (SHREC). Spreading of heterochromatin allows its associated factors to exert influence over extended chromosomal domains.

“Heterochromatin is not a static, inert structure.”

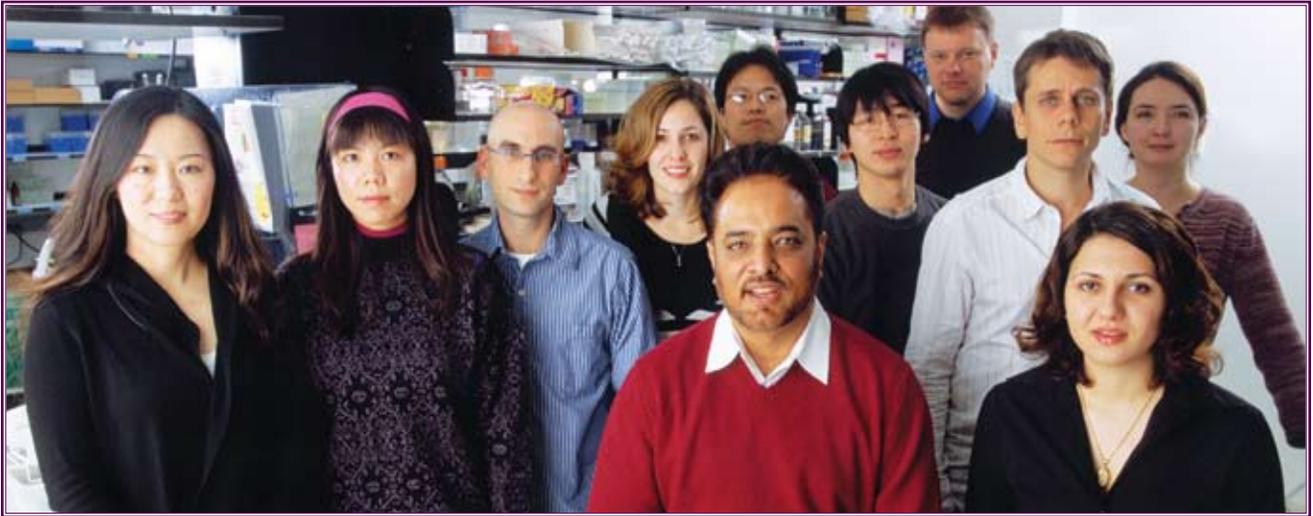
Nature in February 2008 describes the work led by Grewal’s Postdoctoral Fellows Ee Sin Chen, Ph.D., and Ke Zhang, Ph.D., to demonstrate that heterochromatin actually changes during the process of cell division. As cells enter the S or “synthesis” phase, there is a 20-minute window in which DNA at the centromere is actually transcribed, due to a temporary destabilization of the heterochromatin and an active recruitment of the transcriptional machinery involving RNAi complexes. Transcription appears to be necessary in this phase for promoting the proper recruitment of factors controlling heterochromatin silencing during cell division.

In fact, the difference between heterochromatin and euchromatin may actually be a matter of degree—similar mechanisms appear to operate in both, but to different degrees. Recently, Grewal’s lab has been looking at “the rest of the genome” and not just the heterochromatin regions of the telomeres, centromeres, and the *mat* locus. “It is argued that RNAi controls repetitive parts of the genome,”

explained Grewal. However, repeating DNA elements are not restricted to the classic heterochromatic regions. The eukaryotic genome is littered with smaller repeating elements like retrotransposons that were integrated from the genetic material encoded by invading viruses over the course of countless generations. Grewal and his team wanted to know what repressed the expression of these elements.

Postdoctoral Fellow Hugh Cam, Ph.D., led another study that was also published in *Nature* in January 2008 (see “Transposon, Regulate Thyself” in Vol. 2, No. 2 of *CCR connections*). Initially, the team found that a set of proteins in *pombe* related to the human CENP-B protein involved in centromere formation was bound to retrotransposons scattered throughout the genome. Eliminating the genes that code for these CENP-B homologues released these genes from their transcriptional repression. Most interestingly, the CENP-B homologues recruited much of the same machinery to silence gene expression as is found in heterochromatin.

“The cell has a toolkit of repressors [of gene transcription],” concluded Grewal. Silencing of a large chromosomal domain requires a repeat element and RNAi to recruit the repressor complexes associated with heterochromatin and cause it to spread. Now, the same repressors are also silencing other repeat elements in the genome but by a mechanism involving CENP-B homologues that does not cause spreading



(Photo: R. Baer)

Shiv Grewal, Ph.D. (*second from the right in front*), and current members of his laboratory study the mechanisms of epigenetic regulation.

repression. “Again and again, effectors that are on heterochromatin are there on euchromatin, but their targeting differs. If you think in biochemical terms, the differences between heterochromatin and euchromatin are disappearing. It’s really a dynamic balance—in heterochromatin regions, the balance has shifted to more repressors; in euchromatin regions, the balance favors transcription.”

Finding New Challenges

“I knew I wanted to study how chromatin and its complexes modify gene expression with a particular angle to epigenetic regulation, but I didn’t really expect that in 10 years time, we’d know the rough outline of the major pathways,” Grewal said. “The challenge for us is to keep on finding more interesting issues to explore, and so far we haven’t run out.”

Grewal looks forward to the day when he will be able to study chromatin states at the level of a single cell; since most of their work is based on material extracted from populations of cells, information about individual variability is lost. But he knows that it will take some time to get there.

He also believes that new breakthroughs will come from what people currently describe as “unwanted transcription.” DNA is meant to be read by transcriptional machinery in one direction only to produce a functional gene. Two papers have recently appeared, however, reporting that most of the fission yeast genome is transcribed in both directions. “As you look at it more closely, it may have biological implications,” suggested Grewal.

“We are fortunate to be in a lab where we share a lot of interests with the people around us,” Grewal said, pointing to the CCR’s Center of Excellence in Chromosome Biology. He used to feel that his focus should remain exclusively on a deep understanding of the basic mechanisms of epigenetic regulation but senses that the time may soon come to explore further afield. “Being at NCI will provide us with the opportunity to work with other colleagues to apply some of our knowledge. Heterochromatin is at the center of genome stability. What happens in different cancer cell types to heterochromatin structures? Are there ways we can re-engineer these broken

pathways?” Grewal notes that histone deacetylase inhibitors are already used in the treatment of some cancers (see “Histone Modification and Cancer”).

“I’ve been very grateful for the support of CCR’s directors,” concluded Grewal. “Sometimes it’s hard to point out the relevance of these things in the context of cancer, but if you look deeply, you see the implications which are usually borne out with time.”

To read more about Dr. Grewal’s research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=grewal>.

Histone Modification and Cancer

Histone proteins interact with DNA to regulate the structure of chromatin material and the access of machinery to transcribe or silence genes. Enzymes add small molecule groups to these proteins, and these modifications influence this regulation. The most common modifications are the addition of an acetyl group (acetylation) or a methyl group (methylation) to the histone protein. Altered function in both of these processes has been linked to cancer.

The enzyme histone deacetylase (HDAC) removes acetyl groups. In his early work on heterochromatin in fission yeast as a Postdoctoral Fellow, Shiv Grewal, Ph.D., actually worked with HDAC mutants before it was known what they were. All he

knew at the time was that in these mutants (*clr3* and *clr6*), the normal repression of genes in the *mat* locus and centromeres was disrupted. Since then, he and others have shown that HDACs are fundamental to transcriptional repression.

Increasing evidence indicates that the enzymes that regulate histone acetylation—HDACs and histone acetyl transferases (HATs)—are altered in several cancers. Several HDAC inhibitors are currently in various phases of clinical testing for effectiveness as anti-cancer agents (see “Radiating Change,” page 28). Although the mechanism of action is not certain, HDAC inhibitors may relieve the repression of tumor suppressor genes.

The New Face of Pediatric Oncology in Mexico

*An epidemiological transition is taking place in Mexico—pediatric cancers are emerging as one of the leading causes of childhood morbidity and mortality with between 5,000–7,000 new cases diagnosed each year. Ironically, this increase signals Mexico's transition from a developing country in which the childhood diseases of poverty—pneumonia, diarrhea, etc.—were rampant to a society that supports universal access to healthcare for its children; thus, more children survive to the age at which cancers are then diagnosed. However, pediatric oncologists in Mexico are now able to offer a new standard of treatment, which includes the best in medical diagnostics and opportunities to participate in clinical trials of the most promising new drugs. Former CCR Fellow **Aurora Medina-Sanson, M.D.**, now Head of Pediatric Oncology and Hematology at the Hospital Infantil de Mexico Federico Gomez, offers her perspective as someone who has been an integral part of shaping this change by emphasizing the importance of fostering clinical research in parallel with patient care. Part of her inspiration derived from her experiences at CCR.*

Laying the Foundation

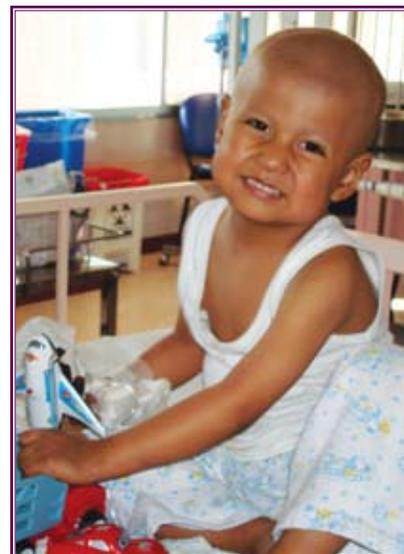
In 1993, I came to the Hospital Infantil de Mexico Federico Gomez for the first time to do a fellowship in pediatric oncology. From the time I started pediatrics training, I knew that I wanted to be a pediatric oncologist. All sick children are special, of course, but I noticed something particular about these children. They really suffer from the treatment, as well as the cancer, but they remain optimistic and happy when they can still retain some ties to normal life. I committed myself to helping them, not only as a physician but also by improving the standard of care we could offer. Of course, at that time, we were in a different world of healthcare in Mexico—before we could even treat these children for their disease, we had to contend with the social issues of where we could find financial support to sponsor their treatment and where they could live during the course of treatment. One of the biggest problems at our hospitals was abandonment; patients simply did not have the resources to complete treatment.

However, even then, I wanted to learn more about the biology of these

cancers and about current research efforts to develop newer and better therapies. In fact, I wanted to start a program of research in Mexico to integrate our large patient base into studies to build new knowledge and contribute to the international effort to solve these diseases. I had met Lee Helman, M.D., Head of the Molecular Oncology Section of CCR's Pediatric Oncology Branch and the current Scientific Director for Clinical Research, on one of his visits to Mexico, and he encouraged me to apply for a grant to do academic research at the NIH. Working in his laboratory at CCR for one and a half years was a transforming experience in my career. It was a turning point in my views of how research is done and on the importance of integrating clinical research into oncology programs. I also learned the value of leadership in a research team—how to develop and translate a vision into an integrated research program while inspiring and trusting your team to be creative and to do remarkable things.

When I came back to Mexico in 2001, I knew we could not begin work on the same scale that I had experienced at the

NIH. But through donations, we procured the equipment to introduce molecular diagnostic techniques—e.g., fluorescent *in situ* hybridization (FISH) and polymerase chain reaction (PCR)—and gradually built up to a formal research program that has been running for the last five years.



(Photo: Courtesy of A. Medina-Sanson)

Pediatric cancer research and treatment are two complementary sides of the same goal: saving children's lives.

A Clinical Research Hub

Hospital Infantil de Mexico Federico Gomez opened in 1943 as the first hospital of Mexico's National Institutes of Health. Our Pediatric Oncology and Hematology Unit receives patients from across the country. We see approximately 300 cases per year, of which 40 percent are leukemia patients, and the rest are solid tumors. Our Laboratory for Research in Hematology and Oncology was formed just two years ago, and it is dedicated solely to research. We have seven pediatric oncologists in our group, all of whom are active researchers. As in the United States, we apply for government and foundation grants to support our research activities.

Every year we take on five to ten fellows in pediatric oncology. Last year, we increased the residency program from two to three years in order to include a research component, and acceptance into the program has become increasingly competitive. Our hope is that we will train future pediatric oncologists to naturally embrace the vision of cancer treatment and research as being two complementary sides of the same goal: saving children's lives.

Mexico, like any other country, has its own unique battles with this disease.

We have several research programs, many in collaboration with other institutions in Mexico and abroad. Currently, we are running approximately 20 investigations, with some of the most important work related to the leukemias that affect the largest proportion of our patients. We are studying the basis of drug resistance that develops in the treatment of acute lymphoblastic leukemia, as well as trying to understand the re-programming events that give rise to the stem cells underlying this disease. Our clinical work also means that we place an emphasis on developing better biomarkers for diagnosis and staging of different cancers, including those for osteosarcoma and retinoblastoma. In addition, cancer immunology and the role of immune status in the development

of cancers are significant parts of our research agenda.

Recently, our hospital became the first Mexican institution to be accepted as a member of the Children's Oncology Group, a worldwide clinical trial cooperative group sponsored by NCI, created with the mission of studying childhood cancers. Through this cooperative, we will be able to participate in and contribute to international multicenter clinical trials for pediatric cancers.

A Focus on Mexico

Cancer is universal, with the same molecular mechanisms at play in people of vastly different genetic and environmental backgrounds. Cancer is also very personal, such that two individuals with seemingly the same disease can respond differently to the same treatment. Cancer is regional as well, and Mexico, like any other country, has its own unique battles with this disease.

Not surprisingly, the dietary habits and available resources of a particular region influence the role of nutrition in pediatric oncology. Thus, our studies of the role of nutrition in pediatric cancers

mean removal of the eyes and even death from disease metastasis. We have several ongoing studies to address retinoblastoma with a particular emphasis on preservation of the eyes—thus far, our success rate for eye preservation has reached close to 90 percent. We are also part of the Mexican Retinoblastoma Group, which aims to create a national registry to better track disease impact and to develop a national treatment protocol.

The last decade has witnessed enormous positive change in the treatment of childhood cancers in Mexico.

Looking Forward

The last decade has witnessed enormous positive change in the treatment of childhood cancers in Mexico. Only 10 years ago, one of our greatest problems was that patients did not have the money to complete treatment. When I came back to Mexico from the NIH in 2001, we still had many difficulties in obtaining the financial resources to treat patients, a common obstacle faced by doctors in developing countries. Now, we have all of the molecular and imaging tools for making a complete diagnosis, our survival scores have improved, and abandonment rates are reduced thanks to changes in our government's policies to completely cover treatment for our children.

Our research program in pediatric oncology and hematology is still relatively young, and so cannot yet be judged by the extent of published results, but we are encouraged by the findings that are beginning to emerge. This year, we will fulfill a longstanding aim of creating a new comprehensive three-floor Pediatric Oncology Unit that should triple our capacity. We are looking forward to the continuing expansion of our research programs and collaborations, both in Mexico and abroad, so that we become equal contributors with institutes like CCR to the international search for cures to childhood cancers.

Radiating Change

Ever since his training at Harvard Medical School, **Kevin Camphausen, M.D.**, knew that his career would combine translational research with patient care. While at Harvard, he trained with C. Norman Coleman, M.D. (now Head of the Radiation Research Program, Extramural NCI), and worked with Judah Folkman, M.D., a pioneer of research into angiogenesis and tumor formation that has resulted in a new class of cancer therapies. As head of the Imaging and Molecular Therapeutics Section in the Radiation Oncology Branch at CCR, Camphausen has a rare opportunity to forge the bench-to-bedside connections that are so vital to the progress of radiation oncology. The branch handles approximately 450 consults per year for many different cancers in adults as well as children. Although his team will do consults and referrals for every patient that comes through the door, Camphausen and his colleagues are limited to treating those that fall within the inclusion criteria of one of their clinical trial protocols. Thus, they cannot treat everyone, but each patient they do treat is also part of research that will lead to a brighter future for others diagnosed with their disease.

Radiation oncology is based on the principle that tumor tissue is more sensitive to radiation damage than normal tissue. Ionizing radiation damages DNA. The same mutations that cause cancerous cells to rapidly proliferate by forfeiting normal cellular checkpoints and DNA repair mechanisms make these cells more vulnerable to the molecular damage inflicted by radiation. In addition, the rapid divisions of cancerous cells cause DNA damage to accumulate at an increasing pace as it is passed on to daughter cells until the progeny are ultimately no longer viable.

Radiation oncology branched off from Radiology as a clinical specialization more than 40 years ago in order to foster its own unique blend of expertise. To treat patients effectively, we have to

understand and manipulate both biology and physics. On the physics side, we use one set of technologies to identify and delineate the tumor within the body—computed tomography (CT) and magnetic resonance imaging (MRI)—and another set of technologies to irradiate it—linear accelerators (Linac). We must decide on the more esoteric parameters controlling the beam of radiation as well as contend with the more mundane but equally challenging issues of making sure the physical placement of the patient (and hence his tumor) in the beam is accurate to within millimeters.

On the biology side, we need to determine which types of tumors are best treated focally and which require wider radiation beams; we need to



(Photo: R. Beyer)

Kevin Camphausen, M.D.

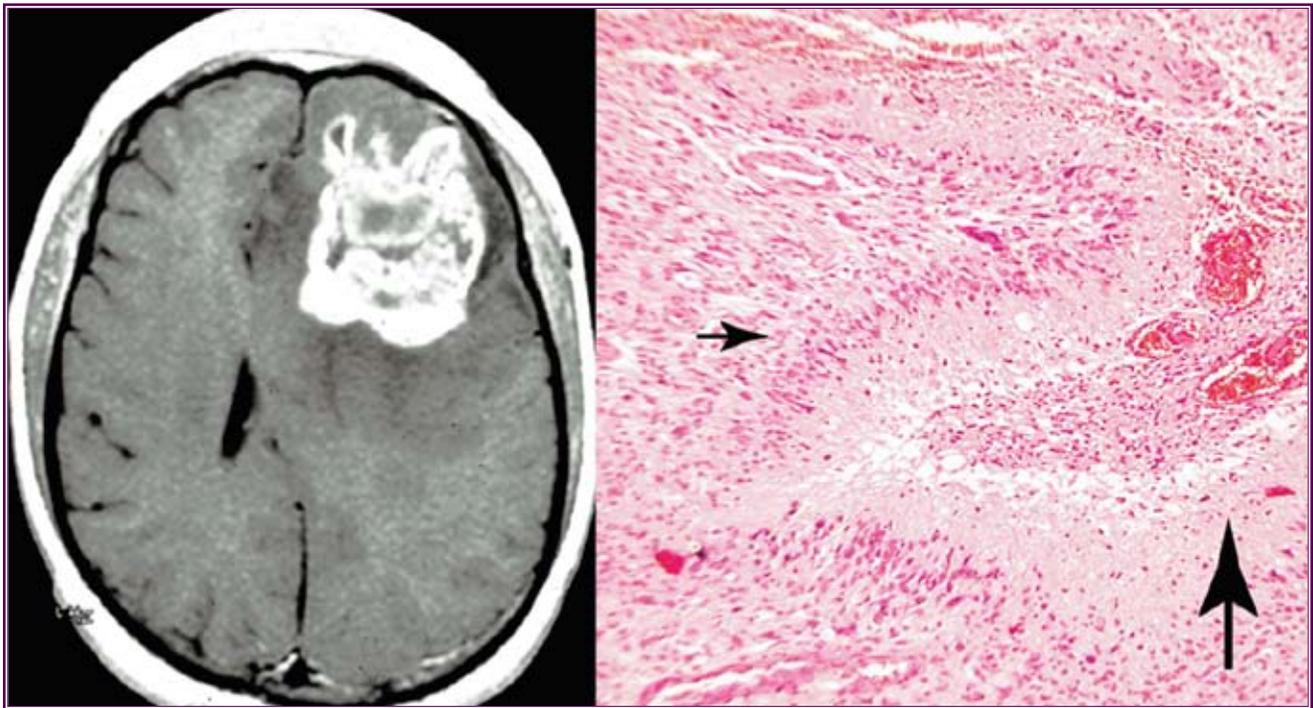
balance treatment between the different sensitivities of normal tissue and tumors. And the greatest potential for advances in radiation oncology lies in a better understanding of tumor biology and in discovering new agents to sensitize cancer cells to radiation.

For me, combining laboratory research, clinical research, and clinical care is the most satisfying way to bring about advances in radiation oncology that will extend and improve patients' lives.

Glioblastoma Multiforme

Although the Radiation Oncology Branch (ROB) is involved in the treatment of a myriad of cancers, my own research focuses on brain cancers. Glioblastoma multiforme (GBM)—a cancerous proliferation of astrocytes, a type of “support cell” in the

The greatest potential for advances in radiation oncology lies in a better understanding of tumor biology.



(Image: Adapted from Weil RJ, 2005: PLoS Med 3(1): e21. doi: 10.1371/journal.pmed.0030031)

Images from a patient with glioblastoma multiforme (GBM). Magnetic resonance image of a large left frontal GBM taken one day before surgery (*left*). Tissue sample from the same tumor showing cellular proliferation and hemorrhage (*right, large arrow*).

brain—is the most common brain cancer with 20,000 new cases diagnosed yearly. This cancer is the same type of cancer that Senator Edward Kennedy was diagnosed with last year. We typically see patients in their 40s and 50s who, after having no previous history of neurological disorders, suddenly experience a seizure or another acute symptom that prompts a physician to order an MRI. It is not uncommon for the GBM to have invaded a large portion of the brain by then.

With the vast amount of information about cancer now available online, most cancer patients tend to be fairly knowledgeable about their disease—my colleague down the hall who specializes in prostate cancer will have patients come in with a three-ring binder full of information that they have downloaded from the Internet about their disease and treatment options. But GBM patients—who may otherwise be in the prime of life with small children under their care—are often shell-shocked. There is not a lot of time between diagnosis and treatment, and the prognosis, unfortunately, is not very good for these patients. The standard of care treatment is a seven-week regimen of radiation therapy in combination with temozolomide, a drug that interferes with

My laboratory has been looking for other drugs that might, in combination with radiation therapy, improve the odds for these patients.

DNA replication. The average length of survival after diagnosis for these patients is 14 months, with about eight months after treatment until signs of disease progression emerge.

My laboratory has been looking for other drugs that might, in combination with radiation therapy, improve the odds for these patients. As a result of the work that we have done in cell and animal models, we are currently running a phase II clinical trial to augment the standard treatment of GBM with the addition of a drug called valproic acid, an inhibitor of the enzyme histone deacetylase (HDAC) (see “Balancing Silence: How a Cell’s Fate Is Determined,” page 22).

From Cell Lines to Human Trials

HDACs are a class of enzymes that are involved in epigenetic regulation—they modify (deacetylate) the histone proteins that in turn interact with DNA to restrict or encourage gene expression. Altered HDAC activity has been seen in several cancers and appears to prevent the expression of tumor suppressor genes, so there has been a great deal of interest in developing HDAC inhibitors as cancer therapeutics.

Some work in the 1980s also indicated that HDAC inhibitors might enhance the sensitivity of tumor cells to radiation, but the available compounds at the time were not suitable for administration to people.

Another scientist here at the NIH, Philip Tofilon, Ph.D. (who has since moved to the H. Lee Moffitt Cancer Center in Florida), had the idea to revisit this therapeutic possibility with the newer generations of HDAC inhibitors that were being developed. In 2004, my team collaborated with Dr. Tofilon to publish research showing that an HDAC inhibitor, MS-275, enhanced the lethal effects of radiation on tumor cells. Unfortunately, we were not able to develop a collaboration with the company that makes MS-275 to continue this line of work, but we were encouraged enough by our results to jump at the suggestion from our colleague, Howard Fine, M.D., Chief of the Neuro-Oncology Branch at CCR, that another HDAC inhibitor—valproic acid—might be an even better choice for enhancing radiation sensitivity. Valproic acid has long been used in the treatment of epilepsy, which means we know it is safe to use in people and will be transported across the barrier that restricts blood-borne molecules from entering the brain.

So we went back and repeated our experiments with valproic acid instead of MS-275. In general, we go through a staged process of testing potential drugs in the laboratory. First, we perform what is known as a clonogenic survival—essentially, we irradiate tumor cells in a dish with or without the compound to see if it affects cell survival. Then we study the cellular mechanisms that might be responsible for the altered survival—regulation of the cell cycle and various cell death programs. Once we are confident that we have a strong result in cell lines, we move to testing animal models. Often, it is sufficient to introduce the cancer cell line of interest under the skin of a mouse and study the resulting tumor formation, but because there are special problems with drugs reaching the brain, my laboratory uses orthotopic models in which a glioblastoma cell line is implanted directly into the mouse brain.

Mouse models are, of course, only models. For example, GBMs in people are highly invasive, whereas they are not in our animal models. And in order to introduce human cancer cell lines into these mice, we need to genetically impair their immune systems so that they do not reject the grafts. However, strong data



(Photo: R. Bate)

Deborah Citrin, M.D., and Kevin Camphausen, M.D., prepare a patient for image-guided radiation treatment in an advanced tomotherapy unit.

that the drug is crossing into the brain and affecting tumors in animal models are usually sufficient to start trials in people.

We are still enrolling patients in our clinical trial for the use of valproic acid to enhance radiation sensitivity in the standard of care regimen for GBM. One challenge that we face is purely practical—unlike many other courses of radiation treatment, the treatment for GBM is protracted. We put the patient on the treatment table every day for a seven-week course of radiation. Thus, it can be difficult to recruit patients who do not live in the vicinity of the NIH.

Advanced Technology

One might imagine that a radiation oncologist could simply use the sophisticated technology at his disposal to visualize the tumor, aim a beam of ionizing radiation at it, and pull the trigger. Unfortunately, the situation is not nearly so straightforward. Instead, the machines that we use to visualize the tumor in the patient's body are distinct from the machines that we use to deliver radiation. Thus, when we physically immobilize the patient in the CT scanner, we use lasers on the wall to place marks on the patient's body so that we know their alignment

with respect to the scanner. We send the patient home, and then we analyze the images and determine the size and position of the beam that we need to use in the subsequent treatment sessions.

Three days later, when the patient is brought in for the radiation treatment, we use another set of lasers to align the marks we made previously and position the patient on the Linac table. We do everything we can to ensure that the patients are placed in precisely the same position every day of their treatment including, for example, the use of frames to constrain head movements, but even a millimeter's difference can affect the targeting of the beam, and this can be especially challenging over the course of a long treatment due to physical changes, such as weight loss, that invariably occur.

New medical technologies are being developed that will make this process less cumbersome and more accurate. Image-guided radiotherapy (IGRT) is emerging as a very precise method of delivering radiation. My colleague, Deborah Citrin, M.D., has a protocol open that is using a tomotherapy unit—a CT scanner that delivers a thousand times higher voltages than those used for diagnostic purposes—allowing us to take very accurate CT

As we are discovering for other cancers, a one-size-fits-all approach to therapy is unlikely to be the answer.

images of the patient and deliver intensity-modulated radiation focally to the tumor. For this particular protocol, she is currently treating patients with metastatic disease outside of the brain, but only in a few tissue sites. The entire course of treatment can be delivered in one week, as compared to the standard seven-week course of radiation.

The Side Effects of Radiation

Although we do our best to irradiate the tumor and spare the healthy cells, cancers are never precisely delineated from their surrounding tissue. Usually, normal tissues can repair the damage caused by radiation, but occasionally these tissues are harmed, resulting in serious side effects. Dr. Citrin has several protocols to assess normal tissue toxicity and to use laboratory methods to predict which patients will experience radiation toxicity.

Most of what we know about radiation damage is from lung cancer. The lung is much easier to study than other organs—X-rays reveal damage more easily, lung function can be measured with a simple pulmonary function test, and the cancer patient population is relatively large. However, different tissues are likely to respond differently to radiation damage. Dr. Citrin is currently conducting a protocol for patients with gastrointestinal malignancies, testing blood, urine, and stool for a wide range of markers of damage and inflammation that may predict malabsorption and other dysfunctions of the gastrointestinal tract.

Cognitive decline is of course a devastating risk of therapies for brain

cancers. I am working with Patricia Steeg, Ph.D. (see “Going after the Real Killer: Metastatic Cancer,” page 12), to study the effects on cognition of radiation therapy for brain metastases from breast cancer. While whole brain radiation therapy can be very effective at destroying these metastases, it is also quite toxic. Through a grant from the Department of Defense specifically aimed at studying brain metastases of breast cancer, we have opened a trial to test prospectively what happens to a patient’s neurocognitive status after whole brain irradiation. Women with breast cancer have typically had a chemotherapeutic agent with its own effects on neurocognition, which has been one of the problems with trying to accurately assess the effects of whole brain irradiation.

Measuring Success

GBM is probably many diseases. We know that the tumors do not all result from the same set of genetic mutations. Because it is a relatively rare disorder, we are only just beginning to gather enough patient data to distinguish subtypes. As we are discovering for other cancers, a one-size-fits-all approach to therapy is unlikely to be the answer.

Beyond subtyping the initial tumors, we are very much interested in finding a way to measure the response to therapy as early as possible. How has the tumor responded to four doses of radiation? Are we having any effect? Can we see any differences in the response to treatment for cases in which the cancer recurs? In our animal models, we biopsy the tumors at regular intervals to test the efficacy of our treatments, but this approach is not an option for human patients.

In collaboration with Marsha Moses, Ph.D., at Children’s Hospital Boston (part of Harvard Medical School), we are studying biomarkers in the urine that might give us some answers. A few years ago, we published some preliminary evidence in the *Journal of Clinical Oncology* that levels of two protein markers of angiogenesis—vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMPs)—might correlate with recurrence of cancer after radiotherapy. Our hypothesis is that these markers reflect renewed tumor growth and the recruitment of new vascular supplies.

Based on this work, we decided to conduct a large clinical trial to assess these urinary biomarkers in GBM patients through the Radiation Therapy Oncology Group—a multi-institutional, international clinical cooperative group funded by NCI. We gathered urine samples from 204 patients with GBM on the first day of treatment, the last day of treatment, and one month later. We will compare the biomarkers with the incidence of recurrence after one year. The data will be unblinded later this year. If successful, these biomarkers could mean being able to treat those patients with a high likelihood of recurrence much more aggressively before it is too late.

To learn more about Dr. Camphausen’s research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=camphausen>.

To learn more about the Radiation Oncology Branch at CCR, please visit <http://ccr.cancer.gov/labs/lab.asp?labid=52>.

One Woman's Story



(Photo courtesy of S. Lassiter)

Sharon Lassiter shows no signs of recurring brain cancer two years after an experimental treatment for glioblastoma multiforme.

Before the headaches started in April 2006, Sharon Lassiter was the picture of health and energy. A mother of two preteens with a full-time position as Deputy Inspector General at Bolling Air Force Base in Washington, D.C., she also found time to be an active member of her church, participate in various clubs, and take spinning classes to keep in shape. "I was go-go-go all the time," she admitted.

Along with the headaches, Lassiter saw flashes in her right eye and developed sensitivity to light, which led the doctors to believe she was having eye migraines. The doctors prescribed migraine medicines, which did not help for very long. "It got to the point that I was wearing sunglasses at work," she said. Then, she started to get dizzy spells and eventually found that she was having more and more difficulty producing the reports that were essential to her job. "I would get home from work and would just go to bed."

As her condition worsened, her eye doctor realized that the problem was neurological and recommended that she have her primary care physician do a computed tomography (CT) scan and use magnetic resonance imaging (MRI). Walter Reed Hospital handled the next phase of her diagnosis and quickly determined that Lassiter had an advanced glioblastoma measuring 3–4 centimeters across in her left occipital cortex. The doctors at Walter Reed sent her to the NIH to determine if she would be eligible to participate in a clinical trial.

"Without treatment, they said I would have 3–4 months to live." The doctors told Lassiter about a trial being conducted by Kevin Camphausen, M.D., to test

the efficacy of valproic acid in addition to the standard of care treatment of radiation and temozolomide after surgery to remove the bulk of the tumor. "I had already prayed about it and decided I would participate," said Lassiter, but she went to see Camphausen with her mother who asked several questions about the prognosis and the treatment. "I liked how they handled it. He explained it in detail and was positive about the possibilities."

Once the treatments started, Lassiter refocused her unflagging energies on the treatment process. "I didn't care what I had to do... The procedure was 10–15 minutes on the table. They made this thing to hold your head down and marked it to make sure they were in the right spot. They played music if you wanted." The staff helped her prepare for the changes she would experience, like the "mental fog" she would feel at the start of the treatment. "Sometimes they were down to the day [in predicting the changes]. It helped that I was prepared."

Lassiter also experienced mild hallucinations from the medication. "There was this lady beside me in the elevator, and I thought she had a beard. I told the doctor, and we laughed about it. He said that was something the valproic

acid could cause, and I shouldn't worry but that I should tell him if the hallucinations got overpowering."

Lassiter went off the medications in November, 2008, and the MRIs she has every three months are tumor free. She was supposed to complete a full two years on the regimen as part of the protocol but found that the side effects were becoming unmanageable. She is, however, participating in another protocol to discover urinary biomarkers that could signal recurrence of the tumor. "I said 'sure'—anything to help someone else with this disease."

As a result of the trauma her brain has suffered, Lassiter has had some loss of vision and experiences problems with balance. The intense lifestyle she once led has given way to a calmer way of living. "To me, that was the hardest part, learning to just take care of me," she noted. "But, now that I've slowed down, I can enjoy my friends and family that much more.

"I have my faith in God, and I know that he's the reason I was able to come to the right place. He blessed my doctors to have the technology, capability, and smarts to be able to do what they do." However, she added, "I do believe that if I didn't want to fight through this, I'd probably be dead."

CCR connections is now available online:
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<http://ccr.cancer.gov>

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<http://ccr.cancer.gov/about/od.asp>

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Office of Training and Education
http://ccr.cancer.gov/careers/office_education.asp

Patient Information on Cancer and Clinical Trials

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<http://www.cancer.gov/clinicaltrials>

How to Refer a Patient
<http://bethesdatrials.cancer.gov/health-care-professionals/index.aspx>

NCI Cancer Information Service
<http://cis.nci.nih.gov>
1-800-4-CANCER (1-800-422-6237)

Understanding Cancer Series
<http://www.cancer.gov/cancertopics/understandingcancer>

CCR Clinical Cancer Trials in Bethesda, Md.
<http://bethesdatrials.cancer.gov>

Additional Links

National Cancer Institute (NCI)
<http://www.cancer.gov>

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