Of Mice and Men:
Tracking the Origins of Metastatic Prostate Cancer
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We invite your comments and suggestions about CCR connections. Please email your feedback to tellccr@mail.nih.gov.

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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.

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Some say that breakthroughs happen when people with a fresh, even naïve, perspective look at an old problem. But in science, particularly in the complex and detail-intense world of biomedicine, sustained work and investment in a particular set of problems, coupled with an open and creative mind, is often the most fruitful path to scientific advancement.

In this issue of CCR connections, we see several examples of researchers that have tackled the hard problems of a particular field for decades and consistently reaped great rewards. In “It’s All About the Client,” we learn how Len Neckers, Ph.D., identified the ubiquitous heat shock protein Hsp90 as an anti-cancer target 15 years ago. Defying conventional wisdom, he worked within NCI to develop the first clinical trial of an Hsp90 inhibitor and paved the way for the 13 Hsp90 inhibitors currently in clinical trials. Neckers and his team are continuing to establish the basic mechanisms of Hsp90 function and doing the preclinical work to optimize this therapeutic strategy.

Along with other NIH intramural colleagues, Gordon Hager, Ph.D., identified the ubiquitous heat shock protein Hsp90 as an anti-cancer target 15 years ago. Defying conventional wisdom, he worked within NCI to develop the first clinical trial of an Hsp90 inhibitor and paved the way for the 13 Hsp90 inhibitors currently in clinical trials. Neckers and his team are continuing to establish the basic mechanisms of Hsp90 function and doing the preclinical work to optimize this therapeutic strategy.

Our feature, “Don’t Throw Out the Packing Materials,” explains how Hager’s work has led to fundamental insights into the dynamic nature of gene regulation that have direct implications for understanding and overcoming the gene dysregulation associated with cancer.

One of our newest tenure-track investigators, Christina Annunziata, M.D., Ph.D., has been studying the subtleties of a single molecular pathway—NF-κB—since she was a graduate student. As an inaugural participant in our Clinical Investigator Development Program, she had the opportunity to apply her insights and knowledge of this pathway from prior research in multiple myeloma and create a strong preclinical ovarian cancer program to evaluate NF-κB pathway inhibitors. “One Molecule, Multiple Cancers: The Devil is in the Details,” describes her plans to translate this research into better treatment for patients.

We are also pleased to have an article from Joyce O’Shaughnessy, M.D., who developed a passion for clinical research in breast cancer during her years at NCI in the late 1980s. She brought that passion with her to Texas Oncology and Baylor College of Medicine, and in “Breast Cancer: The Triple-Negative Problem,” O’Shaughnessy talks about the most recent fruits of her research—results of a very exciting phase 2 trial recently published in the New England Journal of Medicine, on the use of PARP inhibitors for the treatment of triple-negative breast cancer.

At CCR, we are proud of the role we have played in selecting and supporting scientists who have the commitment and fortitude it takes to brave uncharted territories, and who stay the course and get answers from their scientific investigations. Without this kind of sustained effort and determination to grapple with the complex and sometimes very basic questions in biomedical research, clinical breakthroughs would simply not be possible.
Skin Cancer

Interferon-γ has been found to promote UV-induced melanoma in a mouse model.

A series of experiments designed to understand how solar ultraviolet (UV) radiation causes aggressive cutaneous melanoma has led to an unanticipated discovery that the inhibitor of IFN-γ immediately after sunburn might block the carcinogenic activation of melanocytes, the skin’s pigment-producing cells, by UV radiation.

Crucial to the experiments was the development of a unique genetically engineered mouse in which the melanocytes were labeled with a green fluorescent protein. This fluorescent tag allowed melanocytes to be visually tracked and isolated and enabled researchers to evaluate, for the first time, their response to UV radiation while in their natural environment in a living animal.

The researchers observed that UV radiation doses equivalent to those causing sunburn in human skin triggered aberrant growth and migration of melanocytes in mouse skin. UV radiation exposure also persistently activated genes known to respond to IFN-γ, including genes that may help tumor cells evade detection and attack by the immune system. When the activity of IFN-γ was inhibited, the growth and migration of melanocytes remained normal after exposure to UV radiation.

“Interferons have long been touted as anti-tumorigenic and cytostatic—that they have an inhibitory effect on cellular growth,” said Dr. Zaidi.

“The finding that IFN-γ can have a profoundly different effect—that it can exacerbate the growth of melanoma—is a paradigm-shifting discovery.”

The team additionally showed that white blood cells known as macrophages were producing the IFN-γ. Macrophages significantly enhanced melanoma tumor growth when researchers injected them under the skin of healthy mice along with cultured mouse melanoma cells, and this effect was abolished by blocking IFN-γ activity. The researchers also identified IFN-γ-producing macrophages in 70 percent of 27 human melanomas they examined, supporting the possibility that IFN-γ plays a role in this type of cancer—not just in mice, but also in humans.

Moreover, Dr. Zaidi noted, inhibiting IFN-γ immediately after sunburn, an approach that he and his colleagues are pursuing, may prove to be an effective preventive strategy against UV-induced non-melanoma melanoma. The discovery could one day lead to drug treatments that block this mechanism and thus the cancer’s growth, potentially saving many from the lethal threat of skin cancer.

To learn more about Dr. Merlino’s research, please visit his CCR Web site at http://ccr.ncbi.nlm.nih.gov/staff/staff.asp?Name=merlino.

Hitting the Target

Researchers have identified a possible target for treating the most aggressive form of lymphoma.

Single genetic mutations and, more commonly, combinations of mutations lead to the development of cancers such as lymphoma—a cancer of the blood that arises from infection-fighting white blood cells. Diffuse large B cell lymphoma (DLBCL), a type of non-Hodgkin’s lymphoma, is the most common form of this disease and currently has a dismal five-year cure rate. There are three subtypes of DLBCL, of which the activated B cell-like (ABC) lymphoma has the worst outcome with a three-year survival rate of just 40 percent.

However, researchers have identified a recurring genetic mutation that could lead to targeted therapies for ABC lymphoma patients. Mutations of the MYD88 gene (normally involved in the immune response to invading microorganisms) are found in 39 percent of patients with the ABC subtype of DLBCL, and could drive the growth of some lymphoma tumors by activating multiple signaling pathways associated with cancer. A study published in the December 22, 2010, issue of Nature from the laboratory of Louis Staudt, M.D., Ph.D., Deputy Chief of CCR’s Metabolism Branch, reveals a mechanism whereby a single alteration in the MYD88 protein can have a profoundly different effect—that it can exacerbate the growth of melanoma—is a paradigm-shifting discovery.”

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To learn more about Dr. Staudt’s research, please visit his CCR Web site at http://ccr.ncbi.nlm.nih.gov/staff/staff.asp?Name=staudt.

The scientists then looked for specific mutations in MYD88 that might explain the survival-dependence they observed. Sequencing of the MYD88 gene in 352 lymphoma biopsy samples revealed that 29 percent of ABC lymphoma samples had the same mutation, which altered a single amino acid in the MYD88 protein, but this mutation was rare or absent in other lymphoma subtypes. The mutant form of MYD88 sustained the survival of the ABC lymphoma cells while the non-mutated version did not, suggesting that mutations in the MYD88 gene could play an important role in the development of ABC DLBCL.

The researchers then examined proteins that interact with MYD88 in lymphoma cells. The mutant form of MYD88 spontaneously assembled a protein complex that included IRAK1, identified in the genetic screen, and a related protein, IRAK4. In this protein complex, IRAK4 functioned as an enzyme to modify IRAK1, which was required for the mutant MYD88 protein to promote lymphoma cell survival. This particular finding may hold promise for the treatment of lymphomas with MYD88 mutations since pharmaceutical companies are developing IRAK4 inhibitors for use in inflammatory and autoimmune diseases, noted Dr. Staudt.

“The results of this study may provide a method to identify patients with the ABC subtype of diffuse large B cell lymphoma whose tumors may depend upon MYD88 signaling,” said Dr. Staudt. “And these patients may one day benefit from therapies targeting this and other regulatory pathways that sustain the survival of these lymphoma cells.”
Patent Pool Goes Global

CCR-developed HIV drug darunavir is the first patent licensed to the Medicines Patent Pool.

In recent years, there have been heated debates on how to ensure that patents do not stand in the way of access to medicines—especially for the world’s poorest nations. According to UNITAID, an independent global health financing agency founded by the United Nations in 2006, treating a patient for one year with today’s recommended first-line AIDS treatment costs between $151 and $1,033—in part because certain products are patented in some countries. This, according to the agency, is an almost insurmountable problem on its own. With the first license in hand, the Pool is one critical step closer to achieving its goal of making life-saving medicines, like darunavir, more affordable and accessible to people in developing countries.

The Medicines Patent Pool provides a valuable model for attacking a longstanding problem: how to get patented products, developed through public and private investment, to developing countries today without having to wait many years for patents to expire in high-income markets. With darunavir, the NIH demonstrates its commitment to creative approaches to conducting research and development that meet global health needs. Dr. Mitsuya noted, “I am so pleased and honored that darunavir became the first drug to be licensed to the Pool.” Although this is a significant step towards open access to patents for life-saving drugs, this alone will not transform the system unless the pharmaceutical industry follows suit. “The NIH’s move is a good start,” added Dr. Mitsuya, “but this should be only the beginning.”

The Frontiers of Thymic Malignancy

The first International Conference on Thymic Malignancies at the NIH provided a forum to better manage this rare disease.

As every oncologist knows, designing effective treatment and management strategies for individuals with relatively common cancers of the breast or lung is challenging. For rare cancers, where the paucity of cases makes research opportunities scarce, that challenge is magnified enormously. Malignancies of the thymus—a gland that produces and “educates” cells of the immune system called T-lymphocytes—are such rare tumors whose biology is largely unknown. Surgery has been the mainstay treatment in early stages of thymic malignancy, but many patients with advanced stage tumors require aggressive multimodal treatment. Furthermore, the involvement of the thymus results in a broad array of symptoms associated with immune responses and autoimmune diseases. To spearhead further basic and clinical research into thymic malignancies, Giuseppe Giaccone, M.D., Ph.D., Chief of CCR’s Medical Oncology Branch, organized the International Conference on Thymic Malignancies, held on August 20–21, 2009, on the NIH campus in Bethesda, Md. and co-sponsored by the Medical Oncology Branch and the Foundation for Thymic Cancer Research—which brought together more than 100 scientists, pathologists, and clinicians from major institutions in the United States, Europe, and Japan with interest and expertise in the disease and its treatment. The meeting “is one critical step closer to achieving its goal of making life-saving medicines, like darunavir, more affordable and accessible to people in developing countries.”

The first International Conference on Thymic Malignancies speakers’ dinner and reception were held at the Foundation for Advanced Education in the Sciences House [photo: L. Eiben].
A Look at Rare Diseases, from Molecules to Patients

Workshop on Xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy examines diseases of DNA repair, cancer, and premature aging.

Most people do not welcome the signs of aging, but for patients with Xeroderma pigmentosum (XP) and related diseases, the issue is not one of social status but of survival. XP causes patients to develop life-threatening skin cancers and a prematurely aged appearance of sun-exposed skin due to the inability to repair cell damage by ultraviolet (UV) radiation. There are other related disorders such as Cockayne syndrome (CS) and trichothiodystrophy (TTD) that also produce premature aging and are caused by inherited mutations that alter multifunctional protein complexes, which play essential roles in DNA repair and RNA transcription.

These rare diseases have a combined worldwide incidence estimated at less than one per 250,000 births. Yet, there are enough dedicated researchers and clinicians interested in XP, CS, and TTD, and enough cooperative patients and family members, that remarkable progress has been made recently in understanding the molecular basis of these complex disorders. This progress was evident at a workshop—co-organized by Kenneth Kraemer, M.D., Senior Investigator in CCR’s Dermatology Branch; Vilhelm Bohr, M.D., Ph.D., Chief of the National Institute of Aging’s Laboratory of Molecular Gerontology; and Laura Niedernhofer, M.D., Ph.D., Associate Professor at the University of Pittsburgh—to share, consider, and discuss the latest developments in understanding XP and other human diseases characterized by cancer, premature aging, and defects in DNA repair.

The third in a series, this workshop emphasized discussion, interaction, and open exchange of information and ideas among bench scientists, clinicians, patients, and family support group members in both formal and informal settings. “The presence and participation of patients, their advocates, and family support groups was an important and enriching feature of this and the preceding two workshops,” noted Dr. Kraemer. “It is important to emphasize that studying relatively rare diseases such as XP, CS, and TTD may lead to insights that are relevant for more common diseases such as cancer and neurodegeneration.”

For example, XP patients have a 10,000-fold risk of developing new skin cancers and melanomas. “The efforts of all the participants at this workshop may contribute to a greater understanding of rare diseases, as well as a better insight into the risk factors for common diseases in the general population.”

Test Before You Treat

CCR researcher Frank Gonzalez, Ph.D., is recognized for the development of a life-saving diagnostic test to identify cancer patients that may experience 5-fluorouracil toxicity.

In the United States, approximately 275,000 cancer patients receive this drug annually. The transfer of this technology through nonexclusive licenses has enabled the wide dissemination of the diagnostic test. “As a result of these multiple licenses,” noted Gonzalez, “many patients around the world can avoid being treated by a drug that may prove to do them more harm than good.”
new tenure-track scientists

Christina M. Annunziata, M.D., Ph.D.
Christina Annunziata is now a tenure-track investigator in CCR’s Medical Oncology Branch. Her research investigates NF-κB signaling in an ovarian cancer model, and she maintains her clinical focus in the translational clinical studies of ovarian cancer.

Ashish Lal, Ph.D.
Ashish Lal joins CCR’s Genetics Branch. His laboratory focuses on elucidating the function of specific cancer-associated microRNAs using molecular and genetic approaches. His lab is also investigating the role of mutations in tumor suppressor proteins such as p53 on microRNA biogenesis in cancer cells.

Daniel R. Larson, Ph.D.
Dan Larson joins CCR’s Laboratory of Receptor Biology and Gene Expression. His laboratory focuses on the regulation and function of RNA in a cell-biological context, including transcription, splicing, post-transcriptional processing, and decay.

Jayne Stommel, Ph.D.
Jayne Stommel joins CCR’s Laboratory of Biochemistry and Molecular Biology. Her research focuses on oncopgenic kinase signaling in glioblastoma multiforme.

Philip Tofilon, Ph.D.
Philip Tofilon joins CCR’s Radiology Oncology Branch. His research investigates radiation-induced translational control of gene expression, as well as the radiobiology of glioblastoma stem cells.

Christopher Westlake, Ph.D.
Chris Westlake joins CCR’s Laboratory of Cell and Developmental Signaling. His research investigates metastasis trafficking pathways important in clopathy, diseases linked to primary cilia dysfunction, and cancer.

Teri N. Kreisel, M.D.
Teri Kreisel is now a tenure-track investigator in CCR’s Neuro-Oncology Branch. Her research focuses on imaging biomarkers in primary brain tumors.

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2011 Albert B. Sabin Gold Medal Award
Sabin Vaccine Institute

For exemplary contributions to disease prevention through the development or use of vaccines

Douglas R. Lewy, M.D.
Office of the Director, National Cancer Institute

2011 ASCO Pediatric Oncology Award and Lecture
American Society of Clinical Oncology

For outstanding leadership or achievement in the field of pediatric oncology

Lee Helman, M.D.
Office of the Director, Center for Cancer Research

2011 ASCO Statesman Award
American Society of Clinical Oncology

For extraordinary volunteer service, dedication, and commitment to ASCO

Lee Helman, M.D.
Office of the Director, Center for Cancer Research

2011 American Academy of Microbiology Fellow
Michael J. Lichten, Ph.D.
Laboratory of Biotechnology and Molecular Biophysics

2010 AAI Distinguished Service Award
American Association of Immunologists

For outstanding service to AAI through creative and successful initiatives that have benefitted the immunology community

Arthur Andrew Hurwitz, Ph.D.
Laboratory of Human Carcinogenesis

2011 AACR Future Leaders in Cancer Prevention and Epidemiology Research Special Symposium
American Association for Cancer Research

For outstanding early-career scientists in cancer research whose work reflects innovation, scientific independence, motivation, and creativity

Bridgett Ryan, Ph.D.
Laboratory of Human Carcinogenesis

2011 American Academy of Radiology Society’s Failla Award
For a distinguished career in radiation research

James B. Mitchell, Ph.D.
Chief, Radiation Biology Branch

2011 AACR-ACS Award for Research Excellence in Cancer Epidemiology and Prevention
American Association for Cancer Research

For outstanding research accomplishments in the fields of cancer epidemiology, biomarkers, and prevention

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

2011 American Association for the Advancement of Science (AAAS) Award for Excellence in Technology Transfer
Federal Laboratories Consortium Mid-Atlantic Region

For their outstanding work transferring these technologies to the marketplace

A Life-Saving Diagnostic Test for Cancer Patients
Frank J. Gonzalez, Ph.D.
Chief, Laboratory of Metabolism

Novel Protein-Like Therapeutics for the Treatment of Cancer
NadgaTarassova, Ph.D.
Cancer and Inflammation Program

Michael Dean, Ph.D.
Laboratory of Experimental Immunology

Sergey Tarassov, Ph.D.
Structural Biophysics Laboratory

Hong Lou, Ph.D.
Cancer and Inflammation Program

Therapeutic Antibodies for the Treatment of Cancer
Ira Pastan, M.D.
Chief, Laboratory of Molecular Biology

The Presidential Volunteer Service Award
The President’s Council on Service and Civic Participation

Pamela Webb
Pediatric Oncology Branch

The Scientist Magazine: Best Places to Work for PostDocs 2011
 Ranked 14

National Cancer Institute, Bethesda/Frederick

The Scientist: Best Places to Work for PostDocs 2011

Lee Helman, M.D.
Office of the Director, National Cancer Institute, Bethesda/Frederick
In Conversation: Research Fellow Ram Savan, Ph.D.

CCR: Congratulations, Ram, on receiving the Milstein Young Investigator Award from The International Society for Interferon and Cytokine Research last year. We understand that the award honors scientists who have made an impact on interferon or cytokine research early in their careers. Could you tell us about your research?

Ram: I work with Howard Young, Ph.D., Deputy Chief of the Laboratory of Experimental Immunology. The award was for work related to the regulation of INF-γ, which is a focus in our laboratory. We’ve long known that the mRNA for INF-γ is rapidly degraded, almost as fast as it is made. We discovered a microRNA—miR-29—that actually stabilizes the mRNA transcript. From our experiments, we believe that miR-29 binds to the mRNA recruiting a complex that restricts the access of RNA degrading machinery.

CCR: But, aren’t most microRNAs thought to actually help destroy, rather than preserve, gene transcripts?

Ram: Exactly. MicroRNAs recruit RISC complexes. RISC stands for “RNA-induced silencing complex.” When we first observed this microRNA region near the INF-γ gene, we thought it would enhance degradation of the transcript. Imagine our surprise when we added miR-29 and saw more INF-γ than before. We did a lot of different experiments to convince ourselves (and others) that this was real.

CCR: So, you have been working on this project during your 4 years at CCR?

Ram: Yes, but also on two completely different projects. One, which has just been published in Nature, identifies a microRNA-binding regulatory element in the HLA-C gene that influences levels of HLA-C allotype cell surface expression, affecting the immune response to HIV. This project was in collaboration with Smita Kulkarni, Ph.D., and Mary Carrington, Ph.D. The other project looks at a new role for the IL-22 receptor in inflammation, which could be important for certain lymphomas.

CCR: Those are some very diverse projects. How have you done it all?

Ram: Through collaborations, no question. We are part of the Cancer and Inflammation Program, headed by Giorgio Trinchieri, M.D., and it is the most collaborative scientific environment I have ever experienced. It combines two laboratories—ours and the Laboratory of Molecular Immunoregulation—including 15 Principal Investigators. Everyone is incredibly open about their data and keen to help.

CCR: What are your plans for the future?

Ram: I am looking for a faculty position. For the first couple of years, I was learning a new technology and getting unexpected readouts; it was very confusing. So, I created back-up projects and then got very lucky. You don’t want to end up with nothing when a risky project goes wrong.

When I first started thinking about the source of metastases,” said Kelly, “one of the things I found very interesting about the healthy prostate is that when you take away androgens, the prostate shrinks and involutes. And when you add androgen back, it grows.” There aren’t very many dividing cells in the prostate. There aren’t very many cells in the healthy prostate, but manipulations of androgen provided a striking demonstration of the existence and importance of androgen-independent stem cells in the healthy prostate.

“Of the ideas in the field that hasn’t been proven or disproven yet, is that in prostate cancer, an immature undifferentiated cancer cell ultimately gives rise to resistance and metastases,” said Kelly. “The hypothesis is that this cancer stem cell doesn’t require—or has unique mechanisms for obtaining—androgen receptor signaling, so it survives androgen-deprivation therapy.”

In most cases, prostate cancer is a treatable disease. Typically slow growing tumors that occur in men at a median age of 70 years are often treated effectively by interfering with androgen hormone signaling. But in 10 percent of cases, prostate cancers metastasize, become resistant to androgen deprivation therapy, and turn lethal. Kathleen Kelly, Ph.D., Chief of CCR’s Cell and Cancer Biology Branch, has a long-standing interest in understanding the transformation from normal prostate cells into primary cancer and then into metastatic disease. Led by a desire to identify the earliest origins of prostate cancer, Kelly turned to a model system that allows her to study the cells that give rise to the disease as well as trace its metastatic spread.

The Cancer’s Original Sin
Prostate tumors initially require androgen hormone signaling to survive, so androgen-deprivation therapy (ADT), using drugs that inhibit androgen-receptor signaling, has been a highly effective therapeutic option for many patients. Over the years, the drugs that can inhibit androgen receptor signaling have imposed such that the time between when the prostate cancer patient is treated and when he succumbs to the disease has increased. But, when the cancer progresses, it is almost always linked to the development of androgen-independence or “castrate-resistant” prostate cancer. Metastasis is invariably associated with a castrate-resistant form of the disease.

“What I first started thinking about the source of metastases,” said Kelly, “one of the things I found very interesting about the healthy prostate is that when you take away androgens, the prostate shrinks and involutes. And when you add androgen back, it grows.” There aren’t very many dividing cells in the prostate. There aren’t very many dividing cells in the healthy prostate, but manipulations of androgen provided a striking demonstration of the existence and importance of androgen-independent stem cells in the healthy prostate.

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To test the hypothesis, Kelly wanted to look at the cells that initiate prostate cancers and follow their progression.

Building a Better Model
Many human cancers can be effectively studied through xenografts, in which primary tumor cells are injected into mice with compromised immune systems so that they do not reject the foreign cancer cells. Prostate cancer is unusual in that it is extremely difficult to reproduce in such a model system. Furthermore, although they can be kept alive, primary prostate tumor cells do not thrive in culture conditions.

There are a handful of prostate cancer cell lines, which were mostly derived from metastases and not primary tumors, so they have multiple mutations and have been in culture for...
many years. Thus, they are problematic as a tool to study the properties of cancer stem cells, as they might not exist in a living organism.

“The approach I decided to take was a mouse model,” said Kelly. “I chose an aggressive model, because I thought there was a higher chance we could study a metastatic process.”

Kelly and her graduate student, Philip Martin, D.V.M., made a mouse with deletions of two tumor suppressors—PTEN and TP53—in prostate epithelial cells. Mutations of PTEN and TP53 occur at fairly high frequency in human populations and are often associated with aggressive, castrate-resistant, and metastatic disease.

In addition to the two genetic deletions, Kelly and Martin introduced a light-emitting reporter gene—luciferase—which allowed tracking of transplanted cells that carried the genetic deletions.

“The idea we had was that we would be able to sort through the tumor cells and find the tumor-initiating cells in these mice,” said Kelly, “but first we had to fully characterize our model.” So using his training in veterinary pathology, Martin led a full longitudinal study of their mouse model (See “The Veterinary Perspective”).

“One of the most important things we were able to show is that, unlike other mouse models of prostate cancer, this one produced cells with metastatic potential,” said Martin. The mice rapidly developed tumors composed of multiple cell types, which were lethal in approximately seven months.

Panning for Cells

In the Kelly lab, Research Fellow Wassim Abou-Kheir, Ph.D., has studied the progenitor cells undergoing transformation in the prostate of these genetically modified mice. He used selective culturing conditions to study the self-renewing capabilities of prostate cells extracted from these mice. He found that the number of progenitor cells in these mice was strongly amplified and that the cells had a greatly increased ability to self-renew compared to cells from normal mice. “They can be cultured indefinitely and will continue as progenitors. We believe that the tumor-initiating cells are within this self-renewing population,” said Kelly.

Meanwhile, at a nearby bench, Research Fellow Paul Hynes, Ph.D., is searching for tumor-initiating cells by teasing apart identifiable cells from the primary prostate tumors in these mice. The process of fractionating the cells involves separating them out on the basis of protein markers on their cell surface. “It’s finding that there is an undifferentiated tumor-initiating cell that can give rise to both basal and luminal cells,” said Kelly, referring to the two major cell types in the prostate.

The team has also found such bipotential progenitor cells in cell lines that they have created from single tumor cells, i.e., clonal cell lines, and analyzed these cells are also immature, can give rise to both basal and luminal cells, and metastasize when grafted into the mouse prostate.

In 2003, Mark Simpson, D.V.M., Ph.D., Head of the Comparative Molecular Pathology Unit of CCR’s Laboratory of Cancer Biology and Genetics, launched a new training initiative under the NIH Graduate Partnership Program titled the Comparative Molecular Pathology Research Training Program. His aim was to provide opportunities for veterinarians to gain postdoctoral training in pathology and human biomedical research. “The program operates with multiple NIH institute intramural programs and university partners, with training leading to a Ph.D. and eligibility to certify as a specialist in veterinary pathology,” said Simpson. “Training alongside medical and basic scientists fosters a shared vocabulary and approaches to translational research.”

Philip Martin, D.V.M., arrived at the NIH in 2005, part way through a residency in veterinary pathology. “The program was perfect for me because it allowed me to fulfill my residency requirements, including the national board certification exam, as well as pursue a Ph.D., which is important for pathologists who are interested in animal models of human disease,” said Martin.

Martin is currently finishing up his dissertation in the laboratory of Kathleen Kelly, Ph.D., but, in the meantime has accepted a full-time position as a pathologist at CCR’s Center for Advanced Preclinical Research (CAPR) in Frederick, Md. CAPR is dedicated to improving preclinical evaluation for effective cancer diagnosis and treatment.

“If we’re going to study mice as models of human disease, we need to be sure that it is a relevant form of disease,” explained Martin. “The need to analyze the pathology is extremely important. Many models, upon rigorous pathological examination, are not the type of cancer that the investigators were hoping to study.”

“Having Philip’s perspective in the lab has really helped me understand the strong and weak points of mouse models,” said Kelly.

Simpson hopes that this initiative will contribute not only to prevention and treatment of disease in humans, but also in animals. “There’s a special perspective we bring because of our orientation to disease in multiple species.” By integrating the comparative perspective with human biomedical research, Simpson aims to train D.V.M./Ph.D. scientists who are capable of leading research collaborations at the forefront of scientific discovery.

For more information about the Comparative Molecular Pathology Research Training Program, please visit http://ccr.cancer.gov/resources/training/ application/programInformation.asp.
We are really interested in determining how tumor-initiating cells are related to the different cell lineages and then understanding what their response is to androgen and androgen deprivation,” said Kelly.

Kelly and her team have found that some of these clonal cell lines are sensitive to androgen deprivation, but there is a component of cells that does survive androgen deprivation. Kelly noted that in a very recent paper from Memorial Sloan Kettering Cancer Center researchers also found cells from human prostate cancer-derived cell lines that have tumor-initiating capacity and are insensitive to androgen.

“From our mouse work, and now this human cell line, it does appear that a relatively undifferentiated tumor-initiating cell will lead to luminal adenocarcinoma of the prostate,” concluded Kelly.

Understanding more about the cells responsible for driving tumor formation will provide new insights into how to more effectively diagnose potentially aggressive disease and target these specific populations therapeutically.

Tracking the Spread

Another challenge for prostate cancer research lies in the differences in androgen sensitivity that result when cells are removed from the environment of the organism. “It’s complicated to take apart the response,” said Kelly, noting that cells are just not as sensitive to androgen deprivation in culture.

Among the clonal cell lines that Kelly’s team has generated from their mouse model, a few behave like human prostate cancer cells in that they are androgen-sensitive and give rise to adenocarcinoma. “So we are looking at metabolically labeling and tracking these cells in the mouse,” said Kelly.

Peter Choyke, M.D., Program Director of the Molecular Imaging Program at CCR, runs both preclinical and clinical imaging facilities. “Our goal is to develop molecular imaging tools that are translatable into people with the hope that we can diagnose, stage, or monitor cancer patients in a noninvasive way over time,” said Choyke. Among the tools in his research armamentarium is a micro-position emission tomography (PET) scanner for use on small animals.

Choyke and Kelly are planning to use PET to look at metabolic changes in prostate cancer cells relative to normal tissue, both as a tool to better understand prostate cancer progression in the whole organism and as a means to improve the ability to image these cancer cells in men.

“There’s a lot of interest in the metabolomics of cancer and, from an imaging perspective, one of the interesting aspects of prostate cancer is that, early on, it is not particularly well visualized by the standard PET scan,” explained Choyke. Whereas most tumors differenentially depend on glucose uptake, prostate cancers early in development do not, and PET scanning relies on cells taking up radiolabeled glucose (18F-fluoro-deoxyglucose or FDG).

“As the prostate cancer advances and becomes more malignant, it starts to take up more FDG. So, there is some kind of glucose utilization switch that occurs later in its development,” said Choyke.

From studies that Kelly has conducted on prostate cancer cells, she has additional reasons to believe that prostate cancer cell metabolism is altered during the progression of the disease. In addition to changes in glucose metabolism, she believes that changes in fatty acid metabolism might also be important and that early prostate cancers differentially utilize fatty acids. Choyke and Kelly plan to study this by using PET to monitor uptake of the fatty acid precursor, 11C-acetate, which corresponds to the activity of an enzyme that synthesizes fatty acids.

Specifically, Kelly will use a model in which their clonal cell line is introduced into the prostates of mice that have been castrated and implanted with testosterone pellets. Subsequent removal of the pellets will mimic androgen-deprivation therapy and result in the development of androgen-independent malignancies.

“We want to know whether a marker of fatty acid metabolism—11C-acetate—would be a sensitive way of finding prostate cancer cells either in a primary state or following androgen deprivation,” explained Kelly.

One of the biggest challenges to studying and treating any metastatic disease is being able to find and track the cancer as it spreads. Ultimately, Kelly hopes that studying this progression in a carefully characterized and controlled mouse model will provide insights to address that challenge in men.

The Trials of Hsp90

Hsp90 works hard, serving over 200 “client” proteins in the cell, helping them to fold correctly as they take up their rightful positions in the cell. For reasons that are still not well understood, Hsp90 has a special fondness for oncoproteins whose structures shift according to functional state. Among Hsp90’s clients, a surprising number are well recognized targets in oncology, including HER2, a member of the epithelial growth factor receptor (EGFR) family, the fusion protein kinases BCR-ABL and EML4-ALK, the receptor tyrosine kinases KIT and MET, and the steroid hormone receptors for androgen and estrogen. As a result, Hsp90 is predicted to have activity against a variety of cancers.

“Theoretically, heat shock proteins are a very interesting class of drug targets because they are involved in oncogene protein folding in many different tumor types, so their inhibitors should cause downregulation of multiple oncogenes. So, they have the potential to treat different tumor types,” said Giuseppe Giaccone, M.D. Ph.D., Chief of the Medical Oncology Branch at CCR. “And...
Hsp90 inhibitors may be particularly effective in combination with other therapies.

The preclinical data are really incredible—these drugs work at nanomolar concentrations in vitro.”

Because Hsp90 affects multiple oncogenic pathways, Hsp90 inhibitors may be particularly effective in combination with other therapies to lessen development of resistance. “One of the major reasons for drug resistance is the cancer’s resourcefulness. If you shut down one pathway, then a parallel pathway will take over. Hsp90 inhibition could prevent activation of the parallel pathway. So there is a clear role for Hsp90 inhibition in resistant tumors, either upfront to prevent the development of resistance, or later to reverse the resistance once it starts,” said Giaccone. Several clinical trials are under way to test Hsp90 inhibitors in drug-resistant settings.

Neckers agrees that Hsp90 inhibitors could be highly effective in combination with agents targeted against particular oncoproteins. But his research also points to a way forward that utilizes alternative strategies to interfere with Hsp90 in cancer cells. This is important because the first generation of inhibitors have not yet achieved the expected success. “These should be the perfect cancer drugs,” said Neckers, “but so far, the activity in patients is less than you would predict.”

In the Beginning

The Neckers laboratory did not always study Hsp90. “My lab in the late 1980s was working on something completely different: antisense technologies,” said Neckers. However, he was increasingly concerned about off-target effects, that is, interactions that could not be predicted by the sequence of the antisense probe. Neckers had just completed a successful review of his laboratory by the Board of Scientific Counselors, so he felt he had an opportunity to explore new research directions.

Luke Whitesell, M.D., Ph.D., now at the Whitehead Institute for Biomedical Research at M.I.T., was working as a Research Fellow in the Neckers laboratory and had decided to search for novel inhibitors of protein kinases to study their effects on tumor cell morphology. Learning of the antibiotic geldanamycin, isolated from Streptomyces hygroscopicus, that was reported to inhibit the viral oncoprotein v-SRC, Whitesell acquired it and a set of structurally similar compounds called benzoquinone ansamycins from the Natural Products Repository of NCI’s Developmental Therapeutics Program (DTP).

Although the compound did block the ability of v-SRC to transform cells in culture, Whitesell and Neckers soon found that this was not because the compound was directly interfering with the activity of the v-SRC protein. Instead, geldanamycin caused the v-SRC protein to degrade. When they did an experiment to pull out cellular proteins that bound directly to geldanamycin, only one came up—a protein 90 kilodaltons in size, which subsequent experiments confirmed was Hsp90.

“Initially, we thought this was so depressing,” said Neckers. The team was disappointed to pull down a huge amount of what seemed to be a boring cellular housekeeping protein, and nothing else. Fortunately for cancer research, that disappointment didn’t last for long.

“A light bulb went off, and we realized this may be more interesting than we thought. What if this heat shock protein was associating with v-SRC and what if the loss of v-SRC had something to do with the binding of geldanamycin to Hsp90?” asked Neckers.

A series of experiments revealed that geldanamycin bound to an ATP-binding site in the N-domain of Hsp90 and inhibited its ATPase function, that is, the ability of Hsp90 to use energy from ATP to operate as a protein chaperone. Once free of Hsp90, v-SRC became vulnerable to protein degradation machinery in the cell. “That showed us that Hsp90 binds to a client protein and protects its stability,” said Neckers.

Although geldanamycin is itself too toxic to be used in humans, Neckers worked with NCI clinical translation programs—DTP and the Cancer Therapy Evaluation Program (CTEP)—to develop a discontinued compound from Pfizer, 17-AAG, a close chemical relative of geldanamycin, which had been identified in a HER2 inhibitor screen. They tested 17-AAG in preclinical models, verified its Hsp90 inhibitory activity, and began a phase I clinical trial in the late 1990s.

Neckers noted that his former NCI colleague, Edward Sausville, M.D., Ph.D., now at the Greenbaum Cancer Center at the University of Maryland, was instrumental in initiating these clinical trials.

“We found that the less frequently the drug was administered, the higher the doses were that could be tolerated before toxicity became a problem.” At that point, drug companies began to get interested in Hsp90 as a target, considering the ATP-binding site as “druggable.”

Turning to Yeast

Mehdi Mollapour, Ph.D., came to the Neckers laboratory as a Visiting Fellow with a very different background in Hsp90 research. Several years after the Neckers lab
Neither dose was effective in preventing tumor growth in mice, but together, they were highly effective.
Don’t Throw Out the Packing Materials

Most illustrations of DNA depict a kind of ladder spiraling off into the distance, the ladder being the famous DNA double helix consisting of paired nucleotide bases. Although it has long been known that mammalian DNA is packed very tightly and systematically with specialized proteins into material called chromatin, researchers are only now beginning to appreciate the importance of chromatin structure in gene regulation. Gordon Hager, Ph.D., Chief of the Laboratory of Receptor Biology and Gene Expression, has built his considerable scientific achievements on the study of nuclear hormone receptors, using the glucocorticoid receptor (GR) as a prototype. Not without controversy, his research has brought him inexorably closer to the pivotal role and complex dynamics of chromatin structure in the control of gene regulation.

“In the 1980s,” recalled Hager, “chromatin was a bit of a dirty word.” A wave of experiments done by several different laboratories that were designed to transcribe genes from chromatin fractions had ended in the purgatory of experimental artifacts a few years earlier. Wary scientists shied away from studying chromatin as anything more than DNA packing material. “And there were some groups, including mine, Carl Wu’s, Gary Felsenfeld’s, and Bob Simpson’s that did a lot of the early chromatin work because it simply couldn’t get funded extramurally,” explained Hager, referring to his current and former NIH Intramural Research Program colleagues.

Hager’s laboratory was focused on nuclear hormone receptors—receptors that bind hormones like glucocorticoids, which allow them to interact with particular response elements in the DNA to regulate gene transcription. For reasons that are still only partially understood, the murine mammary tumor virus (MMTV) contains a regulatory element that binds GR when MMTV is integrated into cellular DNA. “The team discovered that when MMTV integrated into the mammalian genome, chromatin structural elements called nucleosomes were invariably positioned over the GR binding sites,” Hager said. This discovery was soon followed by studies showing that GR binds directly to a nucleosome and that, as a result, the nucleosome undergoes a structural transition. “To measure the change in chromatin structure, the team used an assay known as DNase hypersensitivity. DNase or deoxyribonuclease is an enzyme that will chew up DNA entirely if incubated long enough. However, when incubated only very briefly, DNA is fragmented at easily accessible sites in the chromatin structure that are termed hypersensitive. GR binding sites corresponded to sites of DNA hypersensitivity. We proposed that the glucocorticoid receptor was binding to DNA and causing chromatin reorganization at a specifically positioned nucleosome,” said Hager.

In 1987, I proposed at a Keystone Meeting in Park City, Utah, that somehow this chromatin reorganization was part of the mechanism by which the glucocorticoid receptor regulated gene expression,” said Hager. Hager was presented with a “Renegade Award” at the meeting, which was not meant as an accolade. “We were in the doghouse,” said Hager. Within a few years, however, the field had shifted its perspective. Evidence began to accumulate suggesting that the structure of chromatin was playing more than just a passive role in gene regulation. A Postdoctoral Fellow in Hager’s lab, Trevor Archer, Ph.D., now Chief of the Laboratory of Molecular Carcinogenesis at the National Institute of Environmental Health Sciences, published an influential experiment in 1992 that presented the first direct evidence that the structure of chromatin could prevent a transcription factor from binding to its promoter element. A few years later, C. David Allin, Ph.D., now Tri-Institutional Professor at The Rockefeller University, and colleagues identified a known transcriptional regulator in yeast as an enzyme that modified histone proteins in chromatin. “And bingo, chromatin was not such a dirty word anymore,” said Hager.

When fluorescent molecules are introduced genetically to label proteins and when the GRs were replaced by green fluorescent protein, they could also study its kinetics through a technique known as photobleaching. When fluorescent molecules are subjected to light of a particular wavelength, they lose their activity and are no longer visible. By shining a laser on the chromosomal segment, Hager’s team could discover whether and when the GRs were replaced by new unbleached molecules. “We found that they were almost instantaneously replaced,” said Hager. “And we were back in the doghouse.”

Not only was the result surprising, the idea that DNA binding proteins were operating on such a fleeting timescale contradicted many accepted views of how DNA binding proteins worked. “We proposed that the glucocorticoid receptor was binding to DNA and causing chromatin reorganization at a specifically positioned nucleosome,” said Hager.

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A Few Years in the Doghouse

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The structure of chromatin was playing more than just a passive role in gene regulation.
experimental paradigms for which protein-DNA binding was essentially considered fixed. However, other groups began to do the same kind of experiments with different proteins and the dynamic nature of these interactions gradually came to be accepted.

Hit and Run

To understand the relationship between GR binding and chromatin remodeling, Hager and his colleagues began to look at chromatin remodeling proteins, massive ATP-dependent chromatin-remodeling complexes that literally grab hold of nucleosomes and alter their higher order structure. Biochemical experiments indicated that the chromatin remodeling protein complex SWI/SNF could create chromatin transitions in the presence of GR. But, they also suggested, paradoxically, that activation of the SWI/SNF complex disrupted GR binding.

Hager’s team decided to use ultrafast UV laser-crosslinking to examine this phenomenon in more detail. They incubated GR and SWI/SNF with chromatin containing the MMTV array of promoter elements and then studied the resulting interactions at different points in time by taking samples and rapidly crosslinking everything with the UV laser. Their results revealed strong evidence that initially, GR binds to the promoter and recruits the SWI/SNF complex, but then is displaced during chromatin remodeling.

“SNF enzymes are giant complexes on cell type. ‘When we compared accessible chromatin regions, we found that the organizational overlap was very, very small. And when we looked at which GR bound in cell lines, very different patterns emerged.’

The convention has always been that GR interactions with chromatin result in hormone-dependent changes in chromatin structure,” said John. “It turns out that the organization of chromatin at baseline is also an important determinant of how a transcriptional regulator finds and binds its target sites in chromatin.”

“By extension, the structure of chromatin at baseline appears to be important for defining a cell,” said John.

DNase hypersensitivity sites—genes—appear to account for 60 to 70 percent of all targets identified in genome-wide disease association studies. That is, many single nucleotide polymorphisms are not located in genes but rather are found in hypersensitivity sites. “This is going to be key in cancer biology—mutations in people that cause dysregulation of their regulatory elements,” concluded Hager.

Far, Far Away

Chromatin structure is not just important within localized regions of DNA. Recently, chromosome conformation-capture technologies are being used to identify distant sequences of DNA that come together when DNA forms loops.

“The best data we have is in T cells, as they mature and differentiate, the whole nuclear material gets reorganized,” said Hager. Genes that are activated come together in clusters, called hubs. “This is the next frontier in cell biology—how to understand the structure of the nucleus. That takes us back to this dynamic question: If the proteins that are binding to these sites are coming and going so fast, how can they possibly get hold of the DNA for long enough to form a long-range interaction?”

“For our group, the dynamics of chromatin remodeling is a key issue. And it all started back in 1987 with that first experiment that put us in the doghouse,” said Hager. “I always wind up at the end of my seminars saying something like ‘biology is chemistry.’ These are chemical reactions, but we often view them as static macromolecular cartoons... The next breakthrough will come from observations of single molecules moving in living cells.”

To learn more about Dr. Hager’s research, please visit his CCR Web site at http://ccr.cancer.gov/staff.asp?Name=Hager.
Joyce O’Shaughnessy, M.D., spent 10 years at NCI, initially as a Clinical Associate, then as a Senior Investigator and Special Assistant to NCI Director, Samuel Broder, M.D., and finally as a Senior Investigator in NCI’s Intramural Breast Cancer Research Program. O’Shaughnessy is now a Medical Oncologist with Texas Oncology and the Baylor-Sammons Cancer Center in Dallas, Texas. She is also Co-Chair of the Breast Cancer Research Program at U.S. Oncology, a practice management company that operates clinical trials across its national network through a structure that resembles NCI Clinical Trials Cooperative Groups. A major focus of her clinical research is on triple-negative breast cancers.

Triple-negative breast cancers are defined by what they are lacking—they do not have the three molecular receptors known as the “holy trinity” of breast cancers: estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2 (HER2). Metastatic, triple-negative breast cancers has a very poor prognosis, with a median survival of only about one year, and there is no standard-of-care therapy.

Triple-negative cancers share similarities with hereditary BRCA1-related breast cancers, namely dysregulation of BRCA1, which leads to defects in repair of double-stranded breaks in DNA. Thus, we and others have wondered whether we could develop therapeutic strategies that exploit this defect in DNA repair. Poly-ADP ribose polymerase (PARP) inhibitors were identified as agents that exploit this defect in DNA repair, thus rendering potentially lethal breast cancers.

The majority of my work involves patients with triple-negative breast cancer because it is such a large unmet medical need. I have developed a particular interest in correlative tissue biomarker studies for triple-negative breast cancer. A few years ago, my colleague Lisa Carey, M.D., and I reported data at the San Antonio Breast Cancer Symposium that the epidermal growth factor receptor inhibitor, cetuximab, showed some activity in breast cancers that are triple negative. We are working on a follow-up study with an intensive biomarker discovery component, in which we hope to understand how to predict the really long benefit—the mutational biomarkers—that we have seen in a subset of patients.

One of the reasons I came to Texas Oncology is because I felt they were very prescient in starting a follow-up study with an intensive biomarker discovery component, in which we hope to understand how to predict the really long benefit—the mutational biomarkers—that we have seen in a subset of patients.

I am definitely encouraged by our advances in the last two decades.

I am involved in a wide variety of clinical trials for high-risk, potentially lethal breast cancers. The majority of my work involves patients with triple-negative breast cancer because it is such a large unmet medical need. I have developed a particular interest in correlative tissue biomarker studies for triple-negative breast cancer. A few years ago, my colleague Lisa Carey, M.D., and I reported data at the San Antonio Breast Cancer Symposium that the epidermal growth factor receptor inhibitor, cetuximab, showed some activity in breast cancers that are triple negative. We are working on a follow-up study with an intensive biomarker discovery component, in which we hope to understand how to predict the really long benefit—the mutational biomarkers—that we have seen in a subset of patients.
One Molecule, Multiple Cancers:
The Devil is in the Details

“Inside the beltway,” is a phrase normally reserved for discussions of careers in national politics, referring as it does to the highway that surrounds the Washington D.C. metropolitan area. However, Christina Annunziata, M.D., Ph.D., has also developed her career as a physician-scientist inside the beltway, first as a medical student and resident at Georgetown University and then rising through the ranks of CCR training opportunities to become a tenure-track Investigator.

Over the years, Annunziata’s responsibilities have involved her in many NCI protocols, but her own research has remained firmly rooted in the family of transcription factors, NF-κB. As a student with Jeffrey Cossman, M.D., at Georgetown, she studied nuclear factor kappa B (NF-κB) signaling in Hodgkin’s disease. As a Medical Oncology Fellow with Louis Staudt, M.D., Ph.D., in CCR’s Metabolism Branch, she studied the role of NF-κB in multiple myeloma. Now her laboratory investigates the effects of NF-κB signaling in ovarian cancer. Annunziata believes that understanding the nuances of NF-κB function in distinct cell types could lead to effective pharmacological interventions for cancer.

It was definitely the opportunity for strong and dedicated research in a clinical environment that drew me to NIH. I met Lou Staudt, when I was still a doctoral student, and it was a natural fit for me to continue my postdoctoral research in his laboratory. NF-κB is a very interesting family of molecules. It consists of five subunits that form various combinations of dimers capable of coordinating the expression of multiple genes. It is present as an inactive form in the cytoplasm of most cells, where a variety of external cellular signals can prompt its rapid separation from a molecular complex and migration to the nucleus to modulate gene expression. NF-κB signaling is important for many different cell types, however, the pathway functions differently according to cell type.

In multiple myeloma, we found that NF-κB signaling was turned on in most of the tumors we studied. In many cases, that may have been the result of influences from the surrounding tissue—the tumor microenvironment—but in some cases, the tumors had autonomous aberrant NF-κB activity. However, we didn’t identify just one specific mutation. We found that there were multiple points throughout the pathway that were dysregulated in different myeloma subtypes. In some cases, a protein might be amplified, in another case a negative regulator might be lost. We did these analyses by first looking at changes in gene expression, but in many cases, validated our findings by looking at protein levels. We believe that one therapeutic action of the protease inhibitor, bortezomib, now approved for the treatment of multiple myeloma, may be to inhibit NF-κB signaling.

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NF-κB Signaling in Ovarian Cancer

While working in the Staudt laboratory, I was performing clinical duties one day per week with Elise Kohn, M.D., in CCR’s Medical Oncology Branch (See “Ovarian Cancer: A Silent Killer “Speaks” through Proteins,” CCR connections Vol. 2, No. 2). I began seeing patients with ovarian cancer, and I began to wonder whether NF-κB signaling might also play a role in this disease. I could not find much in the scientific literature that addressed this question, and I wanted to move my research more towards helping the patients I was actually seeing. So, when I began my research program as an Assistant Clinical Investigator, I began to study NF-κB signaling in ovarian cancer.

My laboratory used a small-molecule drug and small interfering-RNA molecules (siRNA) to inhibit NF-κB signaling in ovarian cancer cell lines. We found that we could define a genetic signature that reflected the upregulation of NF-κB signaling in ovarian cancer and that inhibiting NF-κB signaling affected both this genetic signature and measures of aggressiveness in disease. The signature included genes associated with proliferation, survival, inflammation, adhesion, invasion, and angiogenesis, i.e., all the hallmarks of cancer.

It turns out that NF-κB regulates a very different set of genes in ovarian cancer as compared to multiple myeloma cells. That is hardly surprising, given the very different developmental and functional profiles of the two kinds of cells, but it does mean that the NF-κB activation signature needs to be identified for each cancer type.

We collaborate with Michael Birrer, M.D., Ph.D., who moved from...
Smac, IAP, and Cancer Therapy

Members of the inhibitor of apoptosis protein family (IAP) are exciting targets in cancer research these days. Originally studied for their effects on blocking cell death through inhibition of caspase proteins, IAPs are now known to directly affect multiple cellular processes. Cancer researchers are most interested in the ability of certain IAPs to regulate cell survival and tumorigenesis through activation of NF-κB signaling.

IAPs appear to protect cancer cells from signals related to inflammation in the tumor microenvironment, for example, tumor necrosis factor alpha (TNFα). Alterations in IAPs are found associated with many human cancers and are typically associated with poor prognosis, disease progression, and chemoresistance.

Five biotechnology and pharmaceutical companies currently have early stage clinical trials under way for drugs that interfere with IAPs. These drugs are called Smac mimetics, because they operate like cellular Smac molecules to tap IAPs for destruction. Smac mimetics cause the rapid depletion of certain IAPs and show potent anti-tumorigenic activity in cancer models.

Finding the Right Drug

There aren’t many direct NF-κB inhibitors available, mainly because of issues related to toxicity. So, we’re looking at other points in the pathway that might be more amenable to therapeutic intervention. I am currently working with an investigational drug from Tetralogic Pharmaceuticals, TL-32711, which mimics the cell signaling molecule, Smac. Under certain conditions, Smac shifts the balance of cell signaling from NF-κB-related proliferation to controlled cell death by apoptosis. (See ‘Smac, IAP, and Cancer Therapy’). TL-32711 seems to be more potent and specific than other drugs in its class because of reduced cross-reactivity. We are now studying the effect of Smac mimetics on ovarian cancer cell lines. I also plan to test this drug in mouse models. NF-κB is an important part of normal cellular signaling, particularly in the immune system, which is one reason for the toxicities associated with direct inhibitors of NF-κB. Thus, it will be important to study our Smac mimetic in a mouse model with a normal immune system, so that we can observe the effect of this drug on normal and tumor-infiltrating immune cells. Many mouse models of cancer rely on human cancer cells grafted into a mouse with a deliberately dampened immune system, making such studies impossible.

If, as we predict, Smac mimetics alter expression of the genes we identified as a signature of NF-κB activity, we will want to move our work into human trials for recurrent ovarian cancer. Although such a trial would not be restricted in its enrollment, our hypothesis would be that patients with the highest levels of NF-κB activity would be most responsive to the drug. As the trial proceeds, we would look at the treatment response relative to the level of gene expression from an initial biopsy. In that way, we would hope to home in on the most responsive patient population.

Finding the Right Cancer

I am currently an investigator on about 10 clinical protocols at CCR, at least half of which are for ovarian cancer. Although my research focus is on NF-κB signaling, there are several other candidate targets for this disease including angiogenesis and poly ADP-ribose polymerases (PARPs). Because some ovarian cancers have dysfunction of BRCA1 or 2, including germline mutations, these ovarian cancers have compromised DNA repair. Inhibiting PARP-associated mechanisms of DNA repair is thought to overwhelm the cell’s ability to withstand standard chemotherapeutic agents that disrupt DNA. These were the hot topics at the annual meeting of the American Society of Clinical Oncologists (ASCO) last year, but there are many genetic mutations implicated in this disease, albeit at relatively low frequencies.

A major limitation with all our clinical trials is that we have no way to identify which patients are most likely to respond to a given therapy. Ovarian cancers come in four different histological varieties: serous, clear cell, endometrioid, and mucinous. Interestingly, clear cell ovarian cancer shares some similarity in molecular mutations with clear cell renal cancer and may have a higher response to the drugs sorafenib and sunitinib that are used for renal cancers. So, clear cell ovarian cancer may be a molecular subtype that also has a histological definition. But, that is an exception rather than the rule.

Perhaps even more than in other cancers, it seems that ovarian cancer is extraordinarily heterogeneous at a molecular level. For instance, looking at data on genomic instability from The Cancer Genome Atlas (TCGA), you can see distinct hotspots of genetic abnormalities for glioblastoma and lung cancer patients, but so far, you don’t see that kind of clustering for cases of ovarian cancer. It might be, therefore, that only five percent of ovarian cancers will respond to a particular drug so, without the appropriate molecular testing, clinical trials will continue to see very low response rates.

Our trials are designed so that patients can stay on the therapy as long as there is a response and side effects are manageable. Patients may, in fact, stay on a drug regimen indefinitely. For instance, in the case of the angiogenesis inhibitors, bevacizumab and sorafenib, we’ve had several patients treated for two to three years (See “Warrior Drugs,” page 32). Although there are side effects including high blood pressure and rashes, this drug combination seems to have tolerable levels of toxicity over time, which patients deem an acceptable impact on their quality of life.

There will likely come a point when the tumor evolves to evade a particular drug, and we will have to switch to another drug or another combination of drugs. So, options are important, which is one reason I continue to pursue my work in NF-κB. My personal goal, probably like many of my colleagues here at NCI, is to bring my research into the clinic.
Warrior Drugs

With the right combination of drugs, Patricia Beyea expects to see her ovarian cancer become a managed chronic condition.

Patricia Beyea’s mother died at the age of 52 from breast cancer, so when Patricia felt a lump in her own breast at age 34, she knew it was serious. Ignoring the physician who advised her to wait six months after a negative needle biopsy, she sought another opinion. Shortly thereafter, in 1986, she had a modified radical mastectomy and reconstruction for a breast cancer that had spread to a lymph node.

“That was the first time I saved my own life,” said Patricia.

Given her family history and later discovery that she had inherited a mutation in the BRCA1 oncogene, Patricia has been on a vigilant cancer watch with her doctors ever since. Nine years went by before blood tests revealed the first new sign of cancer. This time, it was ovarian cancer.

“Back then, they were doing in-hospital chemotherapy following surgery,” said Patricia. “A 36-hour treatment with carboplatin and taxol every three weeks—a horrible, tough treatment.” But, the cancer went into remission for another decade.

Patricia retired from her job as a physical education teacher in New York and moved to a small town in Florida. Then, in September 2004, Hurricanes Frances and Jeanne came through, forcing her from her home and into a trailer provided by the Federal Emergency Management Agency (FEMA). “Everything on the outside looked fine, but inside my house was a watermark of 24 inches—sewer water, contaminated water. You had to throw everything out.”

In the busy time that followed, a few things got neglected, including her normal yearly checkup. “All of a sudden, I started having abdominal pains. I went from feeling fine to feeling I couldn’t handle the pain unless I was in a hospital on morphine.”

This time the chemotherapy that followed her surgery was done on an outpatient basis, but that didn’t make it easier to bear. “It’s like you’re dying but you’re still alive,” said Patricia.

The cancer returned twice more after much shorter intervals of remission. After a third recurrence in four years, her surgeon was unable to remove any of the tumor because it was completely surrounded by blood vessels. Patricia could not bear the thought of more standard chemotherapy, which seemed increasingly ineffective.

“I told my oncologist I wanted to enroll in a clinical trial,” said Patricia. “And he asked me where I wanted to go.”

Patricia began her quest for an investigational therapy at M.D. Anderson Cancer Center, but was concerned about signing an agreement making her liable for any costs incurred that were not covered by her insurance. “That’s when the head of the division told me I should self-refer myself to the NIH.”

In September 2008, Patricia enrolled in an NIH protocol for treatment of ovarian cancer with the angiogenesis inhibitors, bevacizumab and sorafenib. She flies from Florida to Washington D.C. every two weeks for the treatments. Her tumors have shrunk in stages and stabilized. “I have finally got to the point of seeing chemotherapy as my ally, not my enemy. Every time I see the drugs going in, I consider them warriors going into battle.”

(Credit: E. Branson)