CCR frontiers IN SCIENCE

July 2002, Volume 1 Published by the Center for Cancer Research, National Cancer Institute

Contents

NATIONAL CANCER INSTITUTE

From the Director

The CCR Office of Training and Education Takes Lead in Offering New Opportunities for Training in Interdisciplinary and Translational Research

Training the next generation of cancer researchers is a critical component of the CCR's mission. Reflecting a major paradigmatic shift in how biomedical research is conducted, future leaders in cancer research will have to develop the collaborative skills necessary to work as part of multidisciplinary teams in order to elucidate the multiple endogenous and exogenous factors—genetic, molecular, environmental, and behavioral—that contribute to human cancers. Moreover, in order to translate basic discoveries quickly into the clinical setting and thereby reduce the burden of cancer on actual human lives, scientists will have to surmount the barriers that have traditionally separated basic researchers, clinical investigators, and medical practitioners.

We established the CCR Office of Training and Education (OTE), headed by Jonathan S. Wiest, Ph.D., to better prepare young postdoctoral fellows to function effectively in this transformed environment. The CCR OTE is now developing numerous new fellowship programs, courses, and career development opportunities that complement those of other NCI offices, most notably the Fellowship Office and the Office of Diversity and Employment Programs. These initiatives also reflect and reinforce the Center's emphasis on promoting interdisciplinary and translational research. Because the CCR encompasses both basic and clinical research programs, it is uniquely positioned to offer young scientists an enriched environment in which they can engage in multidisciplinary collaborations and interact with their clinical counterparts.

The CCR OTE assists in overseeing three Residency and four Clinical Fellowship programs accredited by the Accreditation Council for Continuing Medical Education, five additional Clinical Fellowship Programs, six Translational Research Fellowship programs, and two Basic Science Fellowship programs. Within this framework, Dr. Wiest has been working to facilitate the development of two new fellowship programs designed to build new bridges between the laboratory and the clinic. A Program for Interdisciplinary Training in Chemistry (PITC) is being developed with Chris Michejda, Ph.D. This program aims to attract basic scientists with doctoral degrees in chemistry who are interested in applying their expertise to biomedical research. Fellows will be supervised by two mentors, one chemist and one biologist. PITC is expected to become a mechanism for providing chemistry expertise to such large-scale collaborative efforts within the CCR as the Molecular Targets Initiative. A new translational research fellowship program—the Clinical Cancer Research Fellowship for Ph.D.s—is also being developed in collaboration with Pierre Henkart, Ph.D., to allow fellows to combine their training in basic science with training in cancer medicine and clinical research. As with the chemistry program, each fellow will have two mentors: one in basic science and the other in clinical medicine.

Existing clinical fellowship programs are being enhanced by a new funding mechanism, the Senior Clinical Research Training Awards. Clinical fellowships have generally been limited to three years' duration, with the first year devoted to clinical training and the next two to research. However, it has

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been generally recognized that two years is insufficient time for clinical fellows to mature as researchers. This new award will give fellows an additional two years on campus to develop their research projects.

The CCR OTE also oversees numerous training programs for predoctoral students. It is developing new graduate program partnerships with extramural institutions to address two presently underserved areas of research: bioinformatics and interdisciplinary training in chemistry. The partnership program is intended not only to increase the number of graduate students on campus but also to foster interdisciplinary collaborations. In addition, the OTE serves as a coordinator for the Summer Student Intern Program by placing successful applicants, of which there are approximately 225 each year, with senior investigators who act as their mentors. Howard Young, Ph.D., has developed a series of seminars on selected topics in biomedical research for interns located at Frederick.

Developing new courses designed to foster greater understanding and communication between clinicians and basic scientists is yet another major responsibility of the CCR OTE. The OTE is planning a course on basic molecular biology techniques for M.D.s with Norman Coleman, M.D., as well as a course entitled "Take a Scientist to Clinic," which is intended to teach laboratory scientists about clinical medicine. In collaboration with Dr. Henkart and Irwin Arias, Ph.D., Dr. Wiest has also developed a continuing lecture series entitled "Demystifying Medicine for Ph.D.s," which is now being piloted.

Another vital aspect of the CCR OTE's mission is to offer all postdoctoral fellows a coherent repertoire of career development activities that will help facilitate their transition from mentored to independent research positions and acquaint them with the numerous career pathways open to them. Dr. Wiest has placed great emphasis on developing programs that will give postdoctoral fellows opportunities to hone their scientific writing skills and learn how to compete successfully for grants, most specifically NCI's K22 Career Development Award. He has organized a K22 working group that meets weekly and allows for intensive one-on-one training in grant writing, thereby supplementing the many NIH-wide, one-day writing workshops already in place. Dr. Wiest has also recently created an editorial board consisting of postdoctoral fellows and professional writers who will offer editorial assistance to fellows writing articles for publication. The OTE recently provided support for a course that was recently developed by Leslie Alexandre, Dr. P.H., and Christine Meek, B.A. Entitled "Moving from the Bench into Business: Entrepreneurship in Science," the course teaches the basics of working in the biotechnology industry and launching business ventures.

In partnership with the NCI Fellowship Office, the CCR OTE continues to support the development of the CCR Fellows and Young Investigators Association (FYI) as a forum in which CCR fellows can initiate and pursue their own career development activities. One particularly notable success occurred last year when I challenged the FYI to plan its third annual retreat, a 2-day symposium that attracted world-renowned scientists and gave fellows the opportunity to deliver oral presentations and participate in poster sessions. The fellows organizing the retreat gained valuable administrative experience in planning and running a large scientific meeting: more than 500 fellows and investigators attended the meeting, and 211 abstracts were submitted for review by the planning committee.

Dr. Wiest plans to launch other programs in the near future that will further enhance the postdoctoral experience inside the CCR. Of note, the CCR OTE will offer a new seminar series aimed at preparing fellows for job searches. A retreat for tenure-track investigators is under way. And in conjunction with the Fellowship Office, a fall workshop is being planned to examine interrelations between mentors and postdoctoral fellows and to formulate standards for defining good mentorship.

As a result of the innovative programs now being put into place through the CCR OTE, we believe that future postdoctoral fellows will leave the CCR better trained to engage in collaborative interdisciplinary research, better able to bridge the worlds of the laboratory and the clinic, and better able to compete in our rapidly changing scientific environment. Thus equipped, the recipients of this outstanding training will take the lead not only in clarifying the basic biology of cancer but also in translating future discoveries into new, more effective prevention and treatment modalities.

■ **Carl Barrett, Ph.D.**

■ CLINICAL RESEARCH

Clinical Trial Highlights: Focus on Ovarian and Cervical Cancer

99-C-0137
Samir Khleif, M.D.

A Phase II Study of p53 Vaccine in Patients with Adenocarcinoma of the Ovary

This study will determine whether endogenous cellular immunity to p53 is present in patients with adenocarcinoma of the ovary and whether vaccination with HLA-A2–specific or mutant p53 peptides can induce or boost the immunity of these patients. Patients must have a p53-positive tumor, as shown by immunohistochemical analysis, and no evidence of disease (with or without elevated CA-125) after therapy for initial ovarian cancer. Patients are assigned to a group that receives p53 peptides with GM-CSF subcutaneously or a group that receives p53 vaccine as peptide-pulsed dendritic cells intravenously. Both groups then receive interleukin-2, which enhances the ability of the immune system to kill tumor cells and may interfere with blood flow to the tumor. Treatment is repeated every three weeks for four doses. Patients who show lack of tumor progression or a decrease in CA-125 may continue vaccine treatment for up to two years.

95-C-0154
Samir Khleif, M.D.

A Phase II Study of HPV16 E6 and E7 Peptide Vaccines in Patients with Cervical Cancer

Some types of human papillomaviruses (HPVs) are considered "low-risk" because they rarely develop into cancer. Other types, such as HPV16, are "high-risk" because they can lead to the development of cancer. Proteins produced by HPVs, such as the E6 and E7 proteins of HPV16, interfere with cell functions that normally inhibit growth. This study tests whether endogenous cellular immunity against E6 and E7 is present in patients with advanced or recurrent cervical carcinoma or other carcinomas carrying HPV16. The study then tests whether vaccination with antigen-presenting cells that have been pulsed with synthetic E6 or E7 peptide can induce or boost immunity to that particular peptide. Patients receive the vaccination every three to four weeks for a total of four vaccinations. Treatment continues for a maximum of one year if disease progression or unacceptable toxicity is not exhibited.

00-C-0018
Elise C. Kohn, M.D.

A Pilot Diagnostic Study of Proteomic Evaluation in Patients with Stage III or IV Primary Peritoneal, Fallopian Tube, or Ovarian Epithelial Cancer

Proteomics—the study of proteins inside cells—is generating substantial scientific interest. This study will assess if proteomic technology can reliably detect the relapse of ovarian cancer before symptoms occur. Protein profiles in blood and body fluids are analyzed to identify patterns or changes in patterns associated with relapse and are compared with those expressed in tumor relapse specimens. Patients are seen at baseline, one month, three months, and every three months thereafter for history taking, physical examination, laboratory evaluation, and noninvasive imaging. Patient evaluation continues until relapse is confirmed by biopsy.

01-C-0011
Robert Kreitman, M.D.

A Phase I Study of SS1(dsFv)-PE38(SS1P) Anti-Mesothelin Immunotoxin in Advanced Malignancies: Continuous Infusion x10 Days

This study specifically tests the efficacy of the recombinant immunotoxin SS1(dsFv)-PE38(SS1P) against tumors expressing mesothelin, such as mesothelioma; non-mucinous epithelial ovarian cancer; and squamous cell carcinoma of the lung, head and neck, and cervix. SS1P is composed of an anti-mesothelin variable fragment fused to truncated *Pseudomonas* exotoxin. Patients must have recurrent, unresectable disease after appropriate, definitive therapy.

02-C-0190
Elise C. Kohn, M.D.

A Phase II Trial with Proteomic Profiling of Gleevec™ in Patients with Epithelial Ovarian, Fallopian Tube, and Primary Peritoneal Cancer

Gleevec™, known more formally as STI571 or imatinib mesylate, is FDA-approved for the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumor. This study is the

first to evaluate Gleevec™ as a potential treatment for patients with ovarian cancer. It tests whether a patient's clinical response to the drug Gleevec™ can be correlated with protein changes at a molecular level. Patients receive an oral, daily dose of Gleevec™ for repeated 28-day cycles. A core biopsy of a sentinel or other lesion is obtained upon study entry and at the end of the first cycle. Samples are stored and processed for proteomic pathway profiling. A CT scan to document response is performed at the end of two cycles and every two cycles thereafter. Patients may remain on the study if their disease is stable or if it responds to treatment.

96-C-0011
Patrick Hwu, M.D.

Treatment of Patients with Advanced Epithelial Ovarian Cancer Using Peripheral Blood Lymphocytes Transduced with a Gene Encoding a Chimeric T-Cell Receptor Reactive with Folate-Binding Protein

In this study, patients undergo leukapheresis—removal of blood from the body to collect specific cells, then returning the blood to the body. The specific cells collected for this study are peripheral blood lymphocytes, white blood cells that can destroy tumor cells. The lymphocytes from these patients are expanded in the laboratory, and a gene is inserted so that an anti-ovarian cancer receptor is expressed on their surface. The modified lymphocytes are infused intravenously over 30 to 60 minutes. Patients are immunized with donor blood cells 1 and 8 days after each infusion. If sufficient cells are available, patients may receive subsequent cycles. Follow-up evaluations are done with outpatients approximately one month after each treatment course. Eligible patients must have progressive disease after standard treatment with platinum-based therapy or paclitaxel and tumor tissue available for biopsy to determine antigen expression.

■ IMMUNOLOGY

Self-Sufficient T Cells

Liu K and Rosenberg SA. Transduction of an *IL-2* gene into human melanoma-reactive lymphocytes results in their continued growth in the absence of exogenous IL-2 and maintenance of specific antitumor activity. *J Immunol* 167: 6356-65, 2001.

The Surgery Branch at NCI has pioneered adoptive cell transfer therapy for the treatment of patients with cancer. Identification and transfer of autologous tumor infiltrating lymphocytes (TILs) has mediated objective responses in approximately one-third of patients with metastatic melanoma (Rosenberg SA, *Nature* 411: 380-4, 2001). One major factor limiting the efficacy of this approach is the short survival of these transferred IL-2–dependent lymphocytes. Survival of these highly activated lymphocytes following *in vivo* administration is dependent on an exogenous source of IL-2. Similarly, in the prior studies of cell transfer in murine tumor models developed in the Surgery Branch, the concurrent administration of exogenous IL-2 was essential for the effective elimination of invasive tumors (Rosenberg SA, et al., *Science* 233: 1318-21, 1986). However, the systemic toxicity of IL-2 severely limits the amount of this cytokine that can be given to humans (Rosenberg SA, et al., *Ann Surg* 210: 474-84, 1989).

To overcome this problem and to improve the effectiveness of adoptive cell transfer, Drs. Ke Liu and Steven Rosenberg (Surgery Branch) introduced an exogenous *IL-2* gene into specific melanoma-reactive lymphocytes to enhance their survival and reduce their dependence on exogenous IL-2. They isolated peripheral blood mononuclear cells from a patient immunized with a melanoma-specific peptide vaccine (Rosenberg S, et al., *Nat Med* 4: 321-7, 1998). Following *in vitro* restimulation with the cognate peptide, these cells were transduced with a retrovirus encoding human *IL-2* gene under the

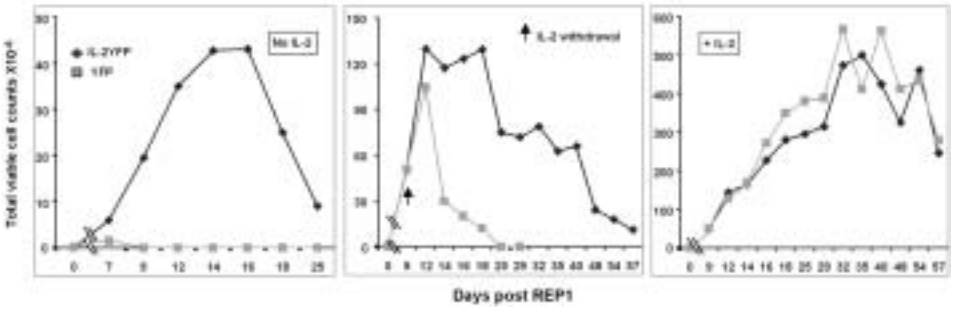

Figure 1: IL-2YFP transductants proliferated in the absence of added IL-2 and maintained their viability after IL-2 withdrawal when they were actively proliferating. IL-2YFP-sorted transductants and YFP-transduced control cells were stimulated with OKT3 and irradiated allogenic feeder cells in a Rapid Expansion Protocol experiment. At the days indicated, viable cell counts were determined by trypan blue exclusion. *Left panel:* no IL-2 from the start. *Middle panel:* IL-2 was added on Day 1 and every 3 days thereafter; then on Day 9, cells were extensively washed and resuspended in complete medium without IL-2 (indicated by an arrow). *Right panel:* IL-2 added from the start (300 IU/ml) and every three days thereafter. Shown here are the results from one stimulation experiment. The growth of the IL-2 transductants could be maintained in the absence of added IL-2 for more than 5 months with subsequent repeated stimulations.

control of the retroviral 5′ LTR promoter. Using a fluorescent marker protein, the IL-2 transductants were subsequently selected by FACS sorting. Unlike cells transduced with a control vector, upon restimulation the IL-2 transductants secreted IL-2, proliferated in the absence of exogenous IL-2, and could be maintained for more than 5 months in the absence of added IL-2 (Figure 1). Moreover, transduction of an *IL-2* gene did not interfere with the high degree of recognition and specificity of transductants against melanoma targets. The continued autocrine growth of these transductants was dependent on restimulation via the T-cell receptor. Thus IL-2-transduced cells may be ideal for adoptive transfer—they can be stimulated and grown only in the presence of tumor.

More recently, using an IL-2 retroviral vector without any marker gene, they achieved gene-transfer efficiencies of TIL cells up to 78 percent. These cells could mediate the destruction of autologous tumors *in vitro* and subsequently proliferate in the absence of exogenous IL-2 while the control vector transductants failed to do so under the same conditions.

The *IL-2* gene-modified lymphocytes described in their study can provide a valuable tool both for *in vitro* studies and for adoptive transfer therapy to patients with melanoma. The principle developed from their study (i.e., sustaining lymphocyte survival while maintaining antitumor activity and specificity by gene modification) may be useful for the immunotherapy of patients with cancer and viral infections. Towards that goal, a clinical protocol has been approved by the NCI Protocol Review and Monitoring Committee and has been submitted to the NCI Institutional Review Board to investigate the fate and therapeutic impact of these *IL-2* gene-modified cells when transferred to patients with metastatic melanoma.

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■ CARCINOGENESIS

Possible Links Between Loss of TGFβ1, Tumor Progression, and DNA Methylation

Yamada H, Vijayachandra K, Penner C, and Glick A. Increased sensitivity of transforming growth factor (TGF) β1 null cells to alkylating agents reveals a novel link between TGFβ signaling and O(6)-methylguanine methyltransferase promoter hypermethylation. *J Biol Chem* 276: 19052-8, 2001.

Both genetic and epigenetic mechanisms contribute to the development of cancer. Genes that regulate the cell cycle and DNA repair are frequently inactivated through the methylation of CpG islands in their promoters. Although methylation occurs frequently in human cancers, questions of timing, mechanism, and causal relationship to specific neoplastic phenotypes are more easily addressed using mouse models. Multistage carcinogenesis of the mouse epidermis is a well-characterized model of squamous cancer in which tumors induced by chemical carcinogens progress at different rates. To determine whether methylation plays an important role in this model of carcinogenesis, we examined the promoter for the DNA repair gene methylguanine methyltransferase *(MGMT)* using a modified PCR-based assay (methylation-specific PCR, or MSP), which can distinguish methylated and unmethylated genomic DNA (Herman JG, et al., *Proc Natl Acad Sci USA* 93: 9821-6, 1996). In human cancers *MGMT* inactivation through hypermethylation may contribute to tumor development through decreased repair of alkylguanine adducts. Our results show that the *MGMT* gene is frequently methylated in both benign and malignant mouse epidermal tumors, and that methylation can be detected in tumors that arise as early as 7 weeks after initiation and promotion (Abdel-Fattah R, in preparation). Although the frequency of methylation generally increased with time and tumor grade, approximately half of the papillomas and carcinomas exhibit *MGMT* methylation, correlating with absence of MGMT protein. These results suggest that in roughly half of the carcinomas that develop in this cancer model, inactivation of the *MGMT* gene could be important for accumulation of mutations and tumor progression. Analysis of other genes in this tumor set will be required to determine if subgroups can be classified according to a methylator or nonmethylator phenotype.

Factors that contribute to altered methylation during cancer development have remained elusive. Perhaps changes in cell cycle regulation, which underlie cancer development, directly or indirectly impact methylation. Transforming growth factor beta (TGFβ) is a member of a large family of multipotent cytokines that play a pivotal role as growth regulators and tumor suppressors for epithelial cells. In the mouse epidermal multistage carcinogenesis model, TGFβ1 acts as a tumor suppressor because progression of chemically induced benign tumors is associated with loss of TGFβ1 (Glick AB, et al., *Proc Natl Acad Sci USA* 90: 6076-80, 1993) and genetic inactivation of the signaling pathway in keratinocytes leads to rapid progression to squamous cell car-

cinoma (Glick AB, et al., *Genes Dev* 8: 2429-40, 1994).

Previously, we reported that nonneoplastic, spontaneously immortal clonal keratinocyte cell lines derived from TGFβ1$^{-/-}$ mouse epidermis had a significantly higher level of gene amplification than similarly derived TGFβ1$^{+/-}$ controls (Glick AB, et al., *Cancer Res* 56: 3645-50, 1996). Surprisingly, all of the TGFβ1$^{-/-}$ cell lines were more sensitive to cell killing by alkylating agents than the TGFβ1$^{+/-}$ lines but were not different in their response to other DNA damaging agents (Yamada H, et al., *J Biol Chem* 276: 19052-8, 2001). Subsequent biochemical and molecular analysis showed that in the TGFβ1$^{-/-}$ cells MGMT was not expressed, and this correlated with methylation of the *MGMT* promoter (Yamada H, et al., *J Biol Chem* 276: 19052-8, 2001). 5-azacytidine restored expression and caused demethylation of the *MGMT* promoter, indicating a causal relationship between methylation and gene inactivation in these cells. Because treatment of the TGFβ1$^{-/-}$ cell lines with TGFβ1 did not cause any change in methylation state, it is likely that hypermethylation is not directly regulated by TGFβ1. However, although the *MGMT* promoter was unmethylated in primary TGFβ1$^{-/-}$ keratinocytes, during prolonged culture and immortalization of these cells (but not the control keratinocytes) *MGMT* promoter methylation increased. The presence or absence of MGMT methylation varied among clonal isolates of the newly immortalized TGFβ1$^{-/-}$ cells, suggesting that a high degree of flux existed in the MGMT methylation state of the TGFβ1$^{-/-}$ cells. Thus absence of autocrine TGFβ1 may predispose keratinocytes to a hypermethylation phenotype, although the exact TGFβ-associated biochemical changes which create this hypermethylation state in the TGFβ1$^{-/-}$ cells are unknown. Because the loss of TGFβ1 expression is associated with malignant progression in the mouse skin tumor model (Glick AB, et al., *Proc Natl Acad Sci USA* 90: 6076-80, 1993) and defects in TGFβ signaling occur frequently in both human tumors and tumor cell lines, we speculate that inactivation of the TGFβ pathway plays a causal role in the widespread hypermethylation of genes that occurs during cancer development and progression.

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■ TRANSLATIONAL RESEARCH

Speeding the Translation of Molecular Oncology into the Clinic

On September 28–29, 2002, Carl Barrett, Ph.D., Director of the CCR, will co-chair the scientific symposium "Molecular Oncology for the Clinical Oncologist" with Larry Norton, M.D., immediate past president of the American Society of Clinical Oncology (ASCO). The symposium, first offered last year in Boston, reflects the latest advances in the field and is the fruit of the longstanding collaborative relationship between the NCI and ASCO. The meeting is an expression of both organizations' strong commitment to encouraging the rapid translation of recent breakthroughs in understanding the basic molecular biology of cancer into the clinical setting.

Genomics and proteomics are revolutionizing approaches to cancer diagnosis, prognostics, staging, therapy, and assessing responses to treatment. For example, microarray analysis provides a new basis for classifying human cancers that will prove to be far more exact than current classifications based upon anatomic location—microarrays will eventually become standard diagnostic tools in the clinic. Moreover, certain patterns of protein expression are now associated with better or worse prognoses. Recognition of the significance of these patterns will eventually allow clinicians to better predict the course of illness in individual patients, thereby giving them a more certain basis for choosing therapeutic options.

The meeting is an expression of both organizations' strong commitment to encouraging the rapid translation of recent breakthroughs in understanding the basic molecular biology of cancer into the clinical setting.

Designed to help clinical investigators expand their knowledge of molecular oncology in order to apply its principles to the design and conduct of clinical trials, the analysis of clinical specimens, and patient care, the symposium has three major foci: molecular diagnosis, molecular staging, and molecular targets of therapeutic agents. Breakout sessions devoted to such topics as specific physiological processes and specific cancers will also be offered. Along with Dr. Barrett, five other NCI researchers have been active as part of the planning committee, and four more are now confirmed to serve as faculty for the symposium. Online registration and housing information are available at http://www.asco.org.

ON THE TENURE TRACK

David Gius, M.D., Ph.D.

Dr. David Gius graduated with a degree in chemistry from the University of Illinois in 1983, received his Ph.D. from the University of Chicago in 1990, and graduated from the University of Chicago's Medical School in 1992. Dr. Gius's residency in radiation oncology was completed at the Mallinckrodt Institute of Radiology, Washington University School of Medicine in 1996. After a 1-year fellowship at the Howard Hughes Medical Institute, he moved to the Institute's Department of Radiation Oncology until the summer of 2001, when he accepted a position at NCI-CCR as head of the Molecular Radiation Oncology Section in the Radiation Oncology Branch.

Dr. Gius

Dr. Gius's research interests revolve around a central hypothesis—that tumor cells use specific intracellular signaling mechanisms to respond to the damaging effects of ionizing radiation (IR). Because irradiation induces both free radicals and oxidative stress, Dr. Gius is investigating the role of redox-sensitive signaling factors in the protection of tumor cells from IR-induced death. He conducts several translational and basic research projects. Overall, his goal is to use discoveries from basic research to determine molecular targets that may sensitize tumor cells to the cytotoxicity of ionizing radiation. He intends to then validate these molecular signatures using a series of molecular biological and biochemical studies. His specific areas of interest are:

- The role of the redox-sensitive signaling factors thioredoxin reductase and thioredoxin as radioprotective factors in cancer cells and their value as molecular targets to sensitize tumors to the cytotoxicity of ionizing radiation.
- The role of the NF-κB transcription factor as a target to alter the inherent radio-responsiveness of cervical tumor cells that don't express p53. This is accomplished by using an agent that prevents the activation of NF-κB by inhibiting proteasome activity.
- The role of epigenetic changes, specifically altered methylation patterns, as a mechanism whereby tumor cells gain a multimodality-resistant phenotype against cytotoxic agents that all appear to induce oxidative stress.

MOLECULAR BIOLOGY

Molecular Anatomy of Prostate Cancer—Assembling Puzzle Pieces

Ahram M, Best CJM, Flaig MJ, Gillespie JW, Leiva IM, Chuaqui RF, Zhou G, Shu H, Duray PH, Linehan WM, Raffeld M, Ornstein DK, Zhao Y, Petricoin EF III, and Emmert-Buck MR. Proteomic analysis of human prostate cancer. *Mol Carcinog* 33: 9-15, 2002.

A primary aim of the Pathogenetics Unit (Laboratory of Pathology) is to help determine the molecular anatomy of prostate cancer; that is, to precisely measure the chromosomal alterations and changes in gene expression and proteomic profiles associated with malignant transformation. Ultimately, it is our goal to synthesize molecular data with clinical information and tumor histopathology to build a comprehensive human disease model useful for the design of diagnostic, prognostic, and therapeutic agents (Cole KA, et al., *Nat Genet* 21: 38-41, 1999; Emmert-Buck MR, et al., *Am J Pathol* 156: 1109-15, 2000; Strausberg RL, et al., *Trends Genet* 16: 103-6, 2000; Emmert-Buck MR, et al., *Mol Carcinog* 27: 158-65, 2000; http://cgap-mf.nih.gov). A recent study by Drs. Mamoun Ahram and Michael Flaig of the Pathogenetics Unit, in collaboration with Dr. Emanuel (Chip) Petricoin of the Food and Drug Administration, illustrates one component of this effort.

To maximize the detection of protein alterations in the tumors, microdissection was performed manually to produce abundant amounts of protein for analysis or by laser capture microdissection to produce highly purified cell samples (Emmert-Buck MR, et al., *Science* 274: 998-1001, 1996). The expression levels of more than 700 proteins were assessed and the identities of 33 proteins determined by mass spectrometry. All 12 of the normal-tumor comparisons shared greater than 96 percent identity homology. However, a small subset (0.25–3.6 percent) of the proteins was altered in the cancer cells of each tumor case when compared to the patient-matched normal prostate epithelium. In total, 40 tumor-specific changes were identified, including proteins involved in morphology (tropomyosin β), metabolism (aldolase A, L-lactate dehydrogenase M chain), and signal transduction (laminin receptor precursor–37 kDa, phosphoserine-threonine-tyrosine interaction protein). Also among the identified proteins were two novel gene products that were downregulated in prostate cancer: protein #36 contains a putative kinase domain homologous to ATP-binding proteins, and protein #47 is homologous to the thioredoxin peroxidase family.

Surprisingly, the tumor-deregulated proteins were almost entirely sample-specific; these proteins were altered in individual tumors, but did not show aberrant expression in any of the other tumors. Only 6 of the 40 proteins showed a change in more than one normal-tumor pair and 5 of these 6 proteins were observed in just two cases. Thus the significance of these protein alterations in prostate cancer is not yet clear. They may represent spurious changes related to tumorigenesis, or they may be useful clues to biochemical pathways deregulated in prostate cancer. Evaluation of additional tumors and follow-up studies of the individual proteins will be necessary to address their potential role in the disease process.

Why were consistent protein changes not observed among the prostate tumors? It may be that prostate tumor biology is exceedingly complex at a molecular level and tumors have few protein alterations in common. Alternatively, uniform changes in protein expression may exist but were not detectable because of their low abundance and/or because current methods for 2D-PAGE protein quantitation cannot detect subtle, yet biologically important, differences in protein levels. Drs. Carolyn Best and Isabel Leiva (Pathogenetics Unit) performed a cDNA microarray-based analysis of moderate- and high-grade prostate tumors to determine whether gene expression changes common to most or all of the neoplasms were present at the mRNA level (Best CJ, et al., manuscript submitted). The expression of 136 genes differed significantly and consistently between tumors and normal samples. This result suggests that common protein alterations among the tumors exist, but are not detectable by 2D-PAGE due to the inherent technical limitations of this methodology. The array data were also amenable to sophisticated statistical analysis performed by Drs. Rich Simon and Yingdong Zhao of the Biometrics Research Branch (Division of Cancer Treatment and Diagnosis). They identified 21 genes that segregate moderate- and high-grade tumors from each other, representing the first time that these clinically important tumor grades have been distinguished by molecular means. In addition to increasing our understanding of the etiology of high-grade prostate cancer, the results of this array analysis may have near-term utility for patient care. Dr. Best has initiated a large clinical-pathological study with Dr. Marek Skacel at the Cleveland Clinic to evaluate the role of the identified genes as prognostic indicators of clinically aggressive prostate tumors.

This proteomic study clearly shows the need for more sensitive and quantitative methodologies for global protein analysis to complement the methods available for mRNA expression studies. The Pathogenetics Unit develops technologies and methodologies that facilitate molecular profiling efforts. These include laboratory methods and strategies for studying mRNA and protein profiles in small numbers of cells, manual and laser-based tissue microdissection techniques, and new tissue processing protocols (http://cgap-mf.nih.gov/Protocols/index.html). In collaboration with Drs. Yingming Zhao and Ge Zhou at the University of Texas Southwestern Medical Center, Dr. Ahram is continuing his proteomic studies of prostate cancer using a new method called differential in-gel electrophoresis (DIGE). This technique is performed by labeling two protein samples (e.g., normal and tumor) with separate fluorescent dyes, running the samples simultaneously on 2D-PAGE, then utilizing a specialized software imaging system that permits quantitative proteome analysis. Preliminary data from three prostate cancer cases suggest that this method characterizes tumor-specific protein alterations better than standard 2D-PAGE. We plan to fully evaluate this new technique by comparing and contrasting the DIGE-derived data with that from the initial 2D-PAGE and cDNA microarray studies. In addition to evaluating the potential of DIGE to improve 2D-PAGE analysis of clinical samples, we are developing a conceptually new technology for proteomic and mRNA-based profiling studies called layered expression scanning (Englert CR, et al., *Cancer Res* 60: 1526-30, 2000; http://www.2020gene.com). This method permits multiplex expression measurements of biological samples (including tissue sections and electrophoresis gels) and could markedly accelerate the identification of molecular targets with clinical impact. In particular, layered expression scanning enables an integrated genomics-proteomics-histopathological analysis of clinical tissue samples. We hope that by using newly developing molecular profiling methods to assemble the expressed pieces of the prostate cancer puzzle, a new picture of this disease will emerge, generating new mechanistic hypotheses, new molecular targets, and ultimately, new treatments for our patients.

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