CCR frontiers IN SCIENCE

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

Contents

NATIONAL CANCER INSTITUTE

From the Director

At this year's CCR-sponsored Fellows and Young Investigators Retreat, held in Baltimore on February 25, retreat planners were pleased that the new NCI Director, Dr. Andrew von Eschenbach, was able to deliver the keynote address. What follows is the basis for his remarks, which he delivered extemporaneously.

It's an honor to join you here this evening. There is nothing more exhilarating than joining this Institute to work with dedicated leaders like Dr. Carl Barrett and talented future leaders like those of you gathered here tonight.

Cancer research is advancing quickly and on many fronts, and we stand at the threshold of a great biomedical revolution. The need for collaboration, for multidisciplinary partnerships, for an interdisciplinary approach has never been more important.

Dr. von Eschenbach

We can no longer live by the "Bench to Bedside" mantra—a linear outlook. The revolution demands a seamless, circular approach that closes the gap between basic researchers, clinical investigators, physicians, nurses, and patients. Individuals can excel, but teams will ultimately succeed.

Our nation's 30-year investment in cancer research has changed the landscape. Every day, laboratories at NIH and around the country uncover another footprint in the process by which a cell becomes malignant, invades, and kills.

While our knowledge of this process is still rudimentary, the path is clear, and greater dividends are within reach. Even with our just emerging picture, we are exploiting this knowledge at every stage in the cancer continuum—with imaging, diagnostic tools, and intervention strategies to treat and prevent cancer.

With a $4.7 billion budget, the NCI must play a lead role in this new paradigm: to maintain momentum, to stimulate new collaborations, to exploit every drop of this wellspring of scientific discovery. That means providing leadership for NCI-funded researchers across the U.S. and around the world, ensuring that results of research are used in clinical practice and public health programs to reduce the burden of cancer, and providing a unique environment that facilitates translational research by providing a defined process for and support to researchers studying promising targeted treatment. In short, an environment for sharing information, informal peer review, and successful collaboration. Just last year, thanks to the vision of Dr. Barrett and others, NCI created just such a diverse, highly interactive environment.

When the two divisions, DCS and DBS, merged in March 2001, a formula for closer links between the lab and the clinical was created. This significantly enhances the interactions and opportunities for both scientific discovery and translational research. With the creation of the CCR, intramural scientists now have an unparalleled environment to move new drugs and diagnostics quickly into clinical studies.

Within this framework emerges one of the most critical planks in NCI's mission: to train you—the 800-strong workforce of fellows and young investigators—to become

CCR frontiers IN SCIENCE

Mission

CCR Frontiers in Science was developed to foster scientific communication within the Center for Cancer Research (CCR) by: 1) promoting awareness of cutting-edge scientific results coming from the Center; 2) fostering scientific collaborations; 3) presenting information on COREs, technologies, and other scientific resources; and 4) providing helpful administrative news briefs with links to corresponding Web sites. Your contributions and comments are welcome. Please send proposals for articles, new ideas, and suggestions to the editor, Sue Fox, by email at smfox@mail.ncifcrf.gov or by telephone at 301-846-1923.

If you have scientific news of interest to the CCR community, please contact one of the Scientific Advisory members responsible for your area, Sue Fox at smfox@mail.ncifcrf.gov, or Tracy Thompson at thompstr@mail.nih.gov.

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the next generation of cancer researchers. It is our responsibility to encourage your growth in the laboratory, in the meeting room, and in the classroom. And it is our responsibility to provide an environment of collaboration, interaction, and translation. The CCR offers numerous predoctoral, postdoctoral, and clinical training positions with world-class scientists and physicians who are outstanding mentors and experts in their respective fields.

We also offer special training awards and other opportunities. For instance, a Senior Clinical Research Fellowship is offered to outstanding clinical fellows to allow a period of intense training in translational research. Areas of partnership currently under development include bioinformatics, chemistry, and comparative pathology.

With these and other tools and resources in place, what do we expect from you? To fully prepare yourself, you will have to work in a way very different from the past. You're going to have to work harder, longer, and smarter than your predecessors. With this new paradigm in mind, I'd like to offer a few suggestions to you:

1. Be proactive in your career and take full advantage of all the educational opportunities available to you. Take advantage of opportunities to learn more, attend courses offered at BIOTRAC or FAES, participate in writing and grantsmanship workshops.

2. Use the scientific resources available to you at NCI—the COREs—the centralized functions for DNA sequencing, microarray expression, and transgenics.

3. Get involved in activities helpful in career development. Look for teaching opportunities. Take opportunities to plan meetings, get involved in planning the FYI retreat. Take time to offer mentoring to summer students and technical support staff.

4. Present your data in posters and in talks every chance you get.

5. Learn and hone the fine art of networking. Use the Web to find other labs doing similar work, both here at NCI, within NIH, and outside of NIH. Get to know the NIH-wide interest groups such as the Proteomics Interest Group or the Mouse Models Interest Group. These listservs span all Institutes and go beyond government. Many biotech companies have joined NIH's listserv interest groups and participate in discussions.

6. Seek out mentors and learn from them—look for people who see your potential and will encourage your growth.

7. Learn the facts about managing your intellectual property. The NIH Technology Transfer Branch is an excellent resource. Learn all you can about intellectual property rights, requirements for disclosures, and ways to get and lend research tools without compromising your right to patents and royalties from new inventions. Understand how to use a Memo of Understanding, Materials Transfer Agreements, and CRADAs.

8. Get to know the breadth and depth of bioinformatics tools available to you. Check out the Cancer Genome Anatomy Project Web site, the proteomics databases, and the cell- or gene-based collections that will serve as ideal tools for your research.

9. Just as important as building on what you already know, get to know an area of research with which you aren't familiar. Use your time at NCI to expand your horizons.

10. And finally, always remember that you are engaged in a pursuit that must transcend its scientific boundaries and reach into the human experience of cancer, the face of cancer, the men, women, and children who each year must battle this devastating disease.

Of course, the responsibility of success at NCI and CCR cuts both ways. Mentoring fellows is and must continue

to be a major goal for the principal investigators in CCR. To help young investigators make the most of the opportunities here and maintain the quality of the candidates applying for training, we need to provide them with an experience that cannot be matched at any other institution in the country.

This can be accomplished by providing the resources, facilities, and education for a prosperous experience, and by interacting with the postdoctoral fellows on a regular basis. Mentors should be proactive by encouraging postdocs to seek out the resources and experiences needed for a successful future career.

Dr. Jonathan Wiest was recruited as the Associate Director of Training and Education to help address these and other issues. His office is a resource for both mentors and fellows to improve the CCR postdoctoral fellowship experience.

We will continue to consider the issues that concern you most: ways that we can increase salaries, tuition loan repayment mechanisms, making the maze of immigration paperwork more reasonable. These messages will be conveyed to the mentors from the leadership of CCR and NCI.

Let me close by saying that this is the greatest of times to be embarking on a career in biomedical research. At the turn of the 20th century, our quest was to understand the fundamental nature of matter. Now, on the threshold of the 21st century, our quest is to understand the nature of life. You have this future—this quest—in your grasp.

IMMUNOLOGY

Overcoming Mechanisms that Downregulate Tumor Immunosurveillance to Improve Cancer Immunotherapy

Matsui S, Ahlers JD, Vortmeyer AO, Terabe M, Tsukui T, Carbone DP, Liotta LA, and Berzofsky J. A model for $CD8^+$ CTL tumor immunosurveillance and regulation of tumor escape by $CD4^+$ T cells through an effect on quality of CTL. *J Immunol* 163: 184-93, 1999.

Terabe M, Matsui S, Noben-Trauth N, Chen H, Watson C, Donaldson DD, Carbone DP, Paul WE, and Berzofsky JA. NKT cell-mediated repression of tumour immunosurveillance by IL-13 and the IL-4R-STAT6 pathway. *Nature Immunol* 1: 515-20, 2000.

Unlike infectious microorganisms, cancer cells are closely related to normal human cells. For this reason, it has been difficult to devise therapies that kill tumor cells without causing substantial side effects to the patient's normal cells. The exquisite specificity of the immune system may offer a way to pinpoint the few unique features of cancer cells and thereby selectively eliminate them. Therefore, immunotherapeutic cancer vaccines have gained great interest in recent years. However, despite limited successes, these vaccines have not yet achieved their anticipated potential. Furthermore, antigenic tumors, which are recognized by antibodies or T cells raised during immunization, often do not induce a sufficient immune response to eradicate the tumor. Nevertheless, immune rejection may occur more frequently than detected, because tumors rejected at a pre-clinical stage may not be recognized. Thus, mechanisms may exist that inhibit natural tumor immunosurveillance and allow clinically significant tumors to arise.

In this context, So Matsui, M.D., Ph.D., and Masaki Terabe, Ph.D., of the laboratory of Jay A. Berzofsky, M.D., Ph.D. (Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch), investigated the mechanisms of tumor immunosurveillance that prevent complete tumor rejection. A fibrosarcoma expressing the HIV-1 envelope protein gp160, which is analogous to the E6 and E7 tumor antigens of human papillomavirus expressed in cervical carcinoma cells, was used as a model of an immunogenic tumor. This tumor grew subcutaneously in BALB/c mice but spontaneously regressed in almost all the mice between 10 and 20 days after tumor implantation. However, around day 30, the tumor recurred and grew uncontrollably in almost every mouse. Mice that had been pre-immunized with a recombinant vaccinia virus expressing gp160 were not completely protected; although initial tumor growth was prevented, tumors still appeared around day 30, at the same time as the recurrence phase in unimmunized mice.

To understand which cells mediated tumor regression and why tumor regression was incomplete, the investigators treated mice with antibodies to deplete $CD4^+$ or $CD8^+$ T cells. Depletion of $CD8^+$ cells completely prevented the initial tumor regression, suggesting that $CD8^+$ T cells were responsible for tumor immunosurveillance. Indeed, mice that hosted tumors, without anti-CD8 treatment, carried $CD8^+$ cytotoxic T lymphocytes (CTL) that could kill tumor cells *in vitro*. Surprisingly, depletion of $CD4^+$ cells did not affect initial tumor growth or regression, but instead prevented tumor recurrence. This finding suggests that a $CD4^+$ regulatory cell prevented complete elimination of the tumor by $CD8^+$ cells, allowing the tumor to escape. In support of this interpretation, mice treated first with anti-CD4, then treated around day 30 with anti-CD8, did not experience tumor recurrence. This result implies that in the absence of $CD4^+$ regulatory cells, tumor elimination mediated by $CD8^+$ cells was complete such that no remaining tumor cells were present to grow, even when $CD8^+$ cells were removed.

To test the hypothesis that Th2 cells, which secrete cytokines IL-4, IL-5, IL-10,

and IL-13, might be the relevant regulatory cells, the investigators studied IL-4 knockout mice, which exhibit markedly reduced Th2 cell activity. Surprisingly, IL-4 knockout mice behaved similarly to wild-type mice: the tumor grew, regressed, and then recurred. However, the tumor failed to recur in IL-4Rα chain knockout mice. The same was observed for mice deficient in STAT6, the downstream signal transducer of the IL-4 receptor. These seemingly paradoxical results implicate IL-13, which is the only other cytokine that uses a signaling pathway involving the IL-4Rα chain and STAT6, in the inhibition of tumor surveillance. To further test this hypothesis, the investigators used an inhibitor of IL-13 consisting of a fusion protein of another high-affinity IL-13 receptor and an immunoglobulin Fc segment. Treatment of the mice with this inhibitor at the time of tumor implantation and for 8 days thereafter prevented tumor recurrence. Therefore, IL-13 appeared to be the primary inhibitor of $CD8^+$ T-cell-mediated tumor immunosurveillance.

Because depletion of $CD4^+$ cells had to be started by day 2 to prevent tumor recurrence, and to determine which cells produce IL-13, the investigators examined other categories of $CD4^+$ cells that are known to secrete cytokines early in the immune response. $CD4^+$ natural killer T (NKT) cells produced much more IL-13 than conventional $CD4^+$ T cells in response to anti-CD3 stimulation. NKT cells are restricted by the non-classical major histocompatibility complex (MHC) class I molecule CD1. Purified $CD4^+$ T cells from tumor-bearing mice, when stimulated *in vitro* with CD1-transfected L cells (compared to control L cells), produced much more IL-13 than did $CD4^+$ T cells from normal mice. In addition, tumors failed to recur and IL-13 production by $CD4^+$ cells decreased greatly in CD1 knockout mice lacking NKT cells. These results indicate that eliminating NKT cells, without eliminating any conventional $CD4^+$ T cells, is sufficient to prevent tumor recurrence and that the critical cell-inhibiting immunosurveillance through the production of IL-13 is the NKT cell. This conclusion is further supported by the detection of CTL activity *ex vivo*, without restimulation *in vitro*, in CD1 mice that lack NKT cells but not in wild-type mice.

IL-13 inhibitors may prove to be a useful tool in cancer immunotherapy.

This study thus defines a new regulatory circuit in which NKT cells, stimulated by the tumor, produce IL-13, which inhibits $CD8^+$ T-cell-mediated immunosurveillance that would otherwise eliminate the tumor. Relieving this inhibition by eliminating the NKT cell, blocking IL-13 activity, or knocking out the receptor or signal transducer for IL-13 could prevent tumor recurrence. Of these options, the one most easily translated to a human clinical trial is the use of the IL-13 inhibitor. Trials are being planned to test an IL-13 inhibitor, either alone as immunotherapy to allow natural immunosurveillance to occur, or as part of vaccine immunotherapy to potentiate the effect of a cancer vaccine.

To complete the regulatory circuit, the investigators are exploring their finding that IL-13 does not directly affect CTL induction or activity *in vitro*, consistent with reports that T cells do not have IL-13 receptors. Therefore, an intermediate cell may respond to IL-13 using the IL-4Rα–STAT6 pathway and, in turn, inhibit CTL. Efforts to identify this intermediate cell and its mechanism of action are currently under way. Current studies are also testing whether this regulatory pathway applies to other tumor models. Preliminary data suggest that it does. Finally, the investigators hypothesize that this same regulatory circuit may dampen CTL responses in other circumstances, such as preventing autoimmune disease; however, vaccine-induced CTL responses may also be inhibited by this mechanism. Therefore, current studies are examining whether blocking this inhibitory pathway will potentiate vaccination against viral infections as well. It is possible that defining this new pathway, which downregulates tumor immunosurveillance, will provide not only new approaches to improve the immunotherapy of cancer, but also new methods to potentiate vaccines against infectious diseases.

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CELL BIOLOGY

SV40 as a Vector for Gene Therapy

Kimchi-Sarfaty C, Ben-Nun-Shaul O, Rund D, Oppenheim A, Gottesman MM. *In vitro*-packaged and SV40 pseudovirions as highly efficient vectors for gene transfer. *Hum Gene Ther* 13: 299-310, 2002.

In collaboration with our colleagues, Ariella Oppenheim, Orly Ben-Nun-Shaul, and Deborah Rund, at the Hadassah Medical Center in Jerusalem, we have been developing the polyomavirus simian virus 40 (SV40) as a vector for gene transfer. In a recent study, SV40 vectors prepared *in vitro* were used for gene delivery. These vectors were prepared, without helper DNA, from recombinant SV40 capsid proteins and plasmid DNA produced in bacteria. Using this system, we have demonstrated efficient delivery of both the human

multidrug-resistance drug transporter gene (*MDR1*), which can confer multidrug resistance on virtually all cell types, and the green fluorescent protein gene (*GFP*) as a reporter. These transferred genes were expressed in human, murine, and monkey cell lines using both cytomegalovirus (CMV) and SV40 promoters. Using a scaled-up protocol for SV40 vector preparation, we have demonstrated highly efficient gene transfer, with essentially 100 percent of transduced cells expressing the transferred gene. The expression of both *MDR1* and *GFP* genes is dose dependent.

SV40 has a small, double-stranded circular DNA genome of 5.2 kb. It also has high transduction efficiency and a broad range of receptive cell types, including bone marrow cells. SV40 binds to its primary receptor, the major histocompatibility complex (MHC) class I (Norkin LC, *Immunol Rev* 168: 13-22, 1999), and has been shown to be nonimmunogenic in mice (Strayer DS and Zern MA, *Semin Liver Dis* 19: 71-81, 1999). Packaging of DNA into SV40 capsids is achieved *in vitro* by incubating the three SV40 capsid proteins—VP1, VP2, and VP3—and agno (produced by baculovirus infection in insect cells) with the plasmid of interest. Plasmids of approximately 8 kb, including approximately 100 bp of the SV40 ori, have previously been shown to be successfully packaged *in vitro* (Sandalon Z, et al., *Hum Gene Ther* 8: 843-9, 1997). In this and other unpublished work, we showed that only VP1 was essential for *in vitro* packaging, that no SV40 sequences were needed for this packaging, and that DNA supercoiled plasmids up to 17 kb could be efficiently packaged and transferred into recipient cells.

We have been developing the polyomavirus simian virus 40 (SV40) as a vector for gene transfer.

The *MDR1* gene encodes a 170-kDa plasma membrane glycoprotein (P-glycoprotein or P-gp). P-gp binds and pumps out structurally diverse compounds and drugs in an energy-dependent manner (Gottesman MM, et al., *Ann N Y Acad Sci* 716: 126-39, 1994). Expression of exogenous P-gp may prevent chemotherapy-induced bone marrow suppression (Aran JM, et al., *Adv Pharmacol* 46: 1-42, 1999). We confirmed that P-gp functions properly by using a Rhodamine 123 fluorescent dye efflux assay and a specific monoclonal MRK16 antibody that detects cell surface expression.

The *in vitro* packaged SV40 vectors were expressed in different cell lines, but the variation in the level of expression suggests that MHC class I receptors play an important role in determining the efficiency of transduction. *GFP* constructs that carried the CMV promoter consistently gave higher expression than those that carried the SV40 promoter. *MDR1* constructs that carried a regulatory element with an intron showed higher expression than those without the intron. In low-expressing MHC class I cell lines, the CMV promoter produced more P-gp expression than the SV40 promoter did.

Both transgenes were only transiently expressed under either the CMV or the SV40 promoter. This is in contrast to pseudovirions prepared with helper virus, whose expression lasted longer (Rund D, et al., *Hum Gene Ther* 9: 649-57, 1998). However, expression of *MDR1* could be maintained for months by selection with colchicine. Transgene expression was rapidly lost when colchicine was removed.

The short-term expression of the SV40/*MDR1 in vitro* vectors may be an advantage for use in chemoprotection. Long-term expression beyond the chemotherapy period is undesirable and may put patients at risk for treatment-induced myelodysplasia or secondary leukemia. The SV40/*MDR1* vectors that are prepared *in vitro* will provide not only a safe vehicle for gene delivery but will also potentially avoid the problem of persistent bone marrow drug resistance in cancer patients.

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RETROVIRUSES

A Role for Inositol Phosphates in HIV-1 Particle Assembly

Campbell S, Fisher RJ, Towler EM, Fox S, Issaq HJ, Wolfe T, Phillips LR, and Rein A. Modulation of HIV-like particle assembly *in vitro* by inositol phosphates. *Proc Natl Acad Sci U S A* 98: 10875-9, 2001.

How is a retrovirus particle assembled from its component parts? The virally encoded Gag polyprotein—the principal structural protein in a retrovirus particle—is sufficient for virus-like particle assembly in mammalian cells. Even in the absence of other viral constituents, viral particles that closely resemble authentic particles are assembled.

Although only the virus-coded Gag protein is necessary for assembly, cellular components could still contribute to the formation of the particle. Alan Rein, Ph.D., and coworkers (Retroviral—Replication Laboratory, HIV Drug Resistance Program) have explored this possibility by using purified human immunodeficiency virus type 1 (HIV-1) Gag protein and an *in vitro* assembly system. In recent years, conditions have been found in which recombinant Gag protein from avian (Campbell S and Vogt

VM, *J Virol* 71: 4425-35, 1997), primate (Klikova M, et al., *J Virol* 69: 1093, 1995), and murine (Campbell S and Rein A, unpublished) retroviruses can assemble into particles similar in size and overall morphology to the parental virus. These "standard assembly conditions" consist of a moderate salt concentration (approximately 0.1M NaCl), neutral pH, and the presence of a small amount of nucleic acid.

These observations suggest that HIV-1 Gag, and perhaps the matrix domain of Gag, binds to IPs or PIPs in vivo.

Although the Gag protein of these viruses is indeed sufficient for particle assembly, in the presence of the right ionic conditions and some nucleic acid, recombinant HIV-1 Gag protein yielded a somewhat different result. It was found that HIV-1 Gag protein assembled into spherical virus-like particles under the standard assembly conditions, but these particles were far smaller than authentic HIV-1 particles—only 25 to 30 nm, rather than approximately 100 to 120 nm, in diameter (Campbell S and Rein A, *J Virol* 73: 2270-9, 1999). In addition, the particles that formed *in vitro* could be disrupted by exposure to a high salt concentration (0.5M NaCl) or RNase, unlike HIV-1 particles produced in human cells.

The observation that particles formed *in vitro* exhibited these differences compared with particles formed in mammalian cells raised the possibility that a factor in the cells altered the process of particle assembly. Dr. Rein's team tested this possibility by supplementing assembly reactions with a mammalian cell lysate. They found that the lysate "corrected" the assembly, resulting in the formation of particles of the correct dimensions and showing resistance to high salt concentration and RNase treatments. One might predict that a protein with a chaperone activity would be involved in facilitating viral assembly. Thus, the fact that fractionation of rabbit reticulocyte lysates ultimately led to the identification of inositol pentakisphosphate (IP5) as the active material in these extracts was rather striking. Further tests have shown that a number of inositol phosphate (IP) and phosphatidyl inositol phosphate (PIP) derivatives can correct the assembly process.

How do IP or PIP compounds facilitate the proper assembly of HIV-1 particles *in vivo*? Remarkably, a single IP5 molecule is sufficient to correct the assembly of approximately 10 HIV-1 Gag molecules. Assembly *in vivo* normally occurs at the plasma membrane; thus, membrane-associated PIP derivatives may well play this role in the cell. The matrix domain of Gag, specifically an N-terminal myristylation and basic residues, confers an affinity to the plasma membranes. Interestingly, a Gag protein in which most of the matrix domain has been deleted (Δ16-99) forms proper particles *in vitro*, in the absence of IPs. These observations suggest that HIV-1 Gag, and perhaps the matrix domain of Gag, binds to IPs or PIPs *in vivo*. It is possible that the presence of the protein in plasma membranes disrupts IP- and/or PIP-based cell signaling pathways, contributing to the cytopathogenicity of HIV-1 infection. Undoubtedly, unraveling the mechanism by which IPs and PIPs facilitate HIV-1 Gag assembly will lead to invaluable insights into this important aspect of HIV-1 replication.

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The Clinical Studies Support Center: A Resource for All of Us

The Clinical Studies Support Center (CSSC) was initially established in 1997 as a call center to answer inquiries about cancer clinical studies taking place on the NIH campus in Bethesda, MD. Since its inception, the Center has answered more than 25,000 calls and developed a comprehensive outreach program to promote NCI intramural research. Patients, family members, physicians, the general public, and NCI clinical staff are all invited to call the CSSC's toll-free telephone service at 888-624-1937, which provides up-to-date information about NCI intramural clinical studies.

The CSSC also has become an integral resource to many NCI investigators and staff who wish to get information about their study options out to interested patients. Several of our principal investigators and research nurses have worked with Center staff to implement outreach efforts to promote their studies, such as creating study-related materials, speaking at area hospitals, and meeting one-on-one with community oncologists. The Center's efforts also encompass outreach to health maintenance organizations, nurses, and special populations, as well as internal NCI staff. These cumulative efforts have had a significant impact on the CSSC's call volume and, ultimately, patient participation in NCI-CCR intramural clinical studies. As a result, the CCR has experienced a 16 percent average increase (per year) in study patients.

The CSSC is a resource for all of us, and we encourage all of our staff members to provide the Center's phone number to physicians, patients, and family members in search of cancer clinical studies. Feel free to call the CSSC and learn more about how they can help you get the word out about your research. If you have questions, contact Deborah Pearson, R.N., B.S.N., Director of Outreach and Patient Recruitment, at 301-435-7854, or call 888-624-1937.

New Mass Spectrometry Center Established at NCI-Frederick

There is a critical need to develop more effective therapies aimed at the prevention or cure of cancer. Because the majority of new anticancer drugs currently under development are targeted toward proteins, it is absolutely imperative to identify those specific proteins that play a key role in cancer manifestation or progression. Mass spectrometry (MS) is now the lead tool for rapid, accurate identification of proteins from both simple and complex mixtures. Working together, NCI and SAIC have brought state-of-the-art MS technology to the NCI via the new Mass Spectrometry Center (MSC) at NCI-Frederick. The MSC is within the Analytical Chemistry Laboratory (ACL), a component of the NCI/SAIC Research Technology Program.

Dr. Tim Veenstra, the Director of the Mass Spectrometry Center, obtained his Ph.D. in biochemistry at the University of Windsor, Ontario, Canada. He then completed his postdoctoral training at the Mayo Clinic Foundation, where he studied the effects of 1,25 dihydroxyvitamin D_3 on gene regulation in neural cells. While at Mayo, Dr. Veenstra used MS to study noncovalent interactions between biomolecules. After his postdoctoral training, he worked as a staff scientist in proteomics at Pacific Northwest National Laboratories. Dr. Veenstra has extensive experience in characterizing proteins using MS, and his strong background in molecular and cellular biology adds to his expertise in designing and implementing complex protein characterization studies.

Using MS to Identify Proteins

The MSC is designed to meet the wide-ranging needs of NCI's intramural community. Currently, the demand ranges from identifying a single protein to determining complex mixtures of proteins. Two primary methods are used: peptide mapping and tandem mass spectrometry (MS/MS).

In both of these methods, the protein(s) of interest is digested into peptides (primarily using trypsin). In peptide mapping, the individual masses of the peptides are measured by the mass spectrometer and, if successful, this collection of masses is mapped back to a single protein. When using MS/MS, a few of the observed peptides are selected for dissociation, so that a collection of fragment masses for each of the selected peptides is obtained. These fragment masses are then used to obtain partial sequence information that may uniquely identify the peptide and hence its protein of origin. Although peptide mapping typically requires three to six peptide masses to match a unique protein for a successful identification, MS/MS can often identify a protein based on the sequence information obtained from a single peptide. The MSC's main focus is protein and peptide characterization; however, the Center also has the capability to characterize small molecules and natural products.

The MSC's instrumentation is well-equipped to perform both peptide mapping and MS/MS and includes:

- four LCQ DECA XP ion traps (sources: electrospray and nanospray ionization)
- one Q-STAR hybrid quadrupole/time-of-flight (sources: electrospray, nanospray, MALDI)
- one Applied Biosystems 4700 Proteomics Analyzer (MALDI-TOF/TOF)
- one SELDI Ciphergen MALDI-TOF

The LCQ DECA XPs are also equipped for automated liquid chromatography (LC)/MS analysis. The 4700 Proteomics Analyzer offers unparalleled mass accuracy for high-throughput MS/MS peptide identification—it can analyze up to 4,000 samples per hour. The SELDI Ciphergen MALDI-TOF is also available for rapid, low-resolution protein profiling.

Because protein identification by MS is directly related to sample quality, the MSC is equipped to handle a variety of sample processing methods (e.g., analysis of gel- and chromatography-separated proteins). The ACL's Separations Technology Group (Haleem Issaq, Ph.D., Head) performs a wide range of chromatographic and electrophoretic separations prior to MS analysis. In addition, MSC staff are experienced in using a variety of stable isotope labeling methods to provide comparative data related to the relative abundance of specific proteins among different samples. The MSC also works with the Advanced Biomedical Computing Center (ABCC; Stan Burt, Ph.D., Director) to analyze and archive MS-related data.

Proteomics Partnerships

The MSC's primary mission will be to partner with the **NCI Clinical Proteomics Program (CPP)** to discover key proteins involved in cancer. The Center will focus on using antibodies and MS to immunoprecipitate and characterize protein complexes. The CPP has established an ever-growing library of high-affinity antibodies directed toward both unmodified and modified versions of important signaling proteins. The MSC will be responsible for identifying as many of the proteins that make up these complexes as possible; this will include site-specific characterization of any posttranslational modifications. Generating a complex series of protein networks will help the CPP identify the key nodes that represent important molecular targets for drug development.

The MSC is also a partner within the **NCI Biomedical Proteomics Program (BPP),** which will assist NCI intramural laboratories in meeting their various protein and mRNA analysis needs. Investigators interested in collaborating

with the BPP will be asked to give a brief presentation of the significance of the proposed study. The BPP will then assist the investigator in designing a research plan that includes the most effective means to complete the project. For more information, visit the BPP Web site at: http://ccr.nci.nih.gov/tech_initiatives/bpp/default.asp.

In summary, the MSC has been designed to meet needs of the NCI intramural community in the areas of sample preparation, mass spectral analysis, and data analysis. For more information, visit the MSC Web site at: http://web.ncifcrf.gov/rtp/labs/ACL/MS/. For descriptions of the laboratories that make up the NCI/SAIC Research Technology Program (RTP), visit http://web.ncifcrf.gov/rtp/.

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On the Tenure Track: Kevin Camphausen, M.D.

In July 2001, Kevin Camphausen, M.D., joined the Radiation Oncology Branch (ROB) as a new principal investigator. Dr. Camphausen majored in biology at Purdue University, proceeded to medical school and internship at Georgetown University, and completed his residency at the Joint Center for Radiation Therapy in Boston in June of 2001. While in Boston he spent 2 years working in the laboratory of Dr. Judah Folkman, where he studied the interactions between radiotherapy and angiogenesis inhibitors. Dr. Camphausen has continued this work here and his lab is currently investigating the mechanisms underlying the synergy between radiotherapy and angiogenesis inhibitors. As head of the imaging and molecular therapeutics section, Dr. Camphausen is developing a translational molecular imaging program with a focus on magnetic resonance (MR), positron emission tomography (PET), and near-infrared optical imaging.

Dr. Camphausen

Dr. Camphausen's clinical responsibilities are focused on prostate and brain tumors. Upcoming prostate clinical protocols will involve MR spectroscopic response to radiotherapy with molecular and proteomic correlates, MR-guided, high-dose rate brachytherapy, and serum/urine protein proteomics. In the brain tumor group, Dr. Camphausen and his colleagues are investigating radiosensitizing agents in pre-clinical models with the goal of translating their results to the design of therapeutic protocols for patients with primary brain neoplasms.

In his personal life, Dr. Camphausen is married; his wife is a physician at Fairfax Hospital, and they have one child who is almost 2 years old. Dr. Camphausen and his family live in northern Virginia, where he tries to spend as much time as possible outdoors playing with his son.

Administrative Links

NIH Commissioned Corps Honors
The Commissioned Corps (CC) Honor Awards are presented annually to NIH Commissioned Officers whose achievements and/or services warrant special recognition. Nominations will be accepted for all CC awards twice this year. The deadline for submission of names for the first cycle was March 15th, and the deadline for the second cycle is July 15th. Nominations should be forwarded to the NCI Human Resource Management and Consulting Branch (HRMCB) 2 weeks before these dates. To see the full story, click on http://camp.nci.nih.gov/admin/news/admin/200203/nominations.htm.

To learn more about NIH awards, go to http://www1.od.nih.gov/ohrm/Awards/.

The HRMCB Homepage can be found at http://camp.nci.nih.gov/public/hr/index.html.