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The recruitment of established senior investigators can have a profound impact on an institution. Over the course of the last three years, the Robert H. Lurie Comprehensive Cancer Center of Northwestern University has been fortunate to welcome a number of highly regarded clinicians and scientists to our Cancer Center. These individuals are listed by year with their previous institution, Cancer Center program affiliation and research area.

2001

Thomas Adrian, PhD – Creighton Cancer Center, Creighton School of Medicine; Gastrointestinal Oncology Program; Growth factors and intracellular signaling in pancreatic cancer.

Martha Bohn, PhD – University of Rochester Medical Center; Cancer Genes and Molecular Regulation Program; Neurotrophic factors and gene therapy for neurodegenerative diseases.

Richard Carthew, PhD – University of Pittsburgh; Hormone Action and Signal Transduction in Cancer Program; Molecular mechanisms that receptor tyrosine kinases use to regulate cell behavior.

2002

Jayesh Mehta, MD – South Carolina Cancer Center and Palmetto Richland Memorial Hospital; University of South Carolina; Hematologic Malignancies Program; Bone marrow and stem cell transplantation.

Seema Singhal, MD – South Carolina Cancer Center and Palmetto Richland Memorial Hospital; University of South Carolina; Hematologic Malignancies Program; Plasma cell dyscrasias.

Thomas Meade, PhD – California Institute of Technology; Cancer Genes and Molecular Regulation Program; Inorganic coordination chemistry for the study of molecular imaging in vivo gene expression.

Leonidas Platanias, MD, PhD – University of Illinois at Chicago; Hematologic Malignancies Program; Mechanisms of signal transduction of interferons and other cytokines in malignant cells.

Gustavo Rodriguez, MD – Duke University Medical Center; Gynecologic Oncology and Hormone Action and Signal Transduction Programs; Effective pharmacologic approach for the prevention of ovarian cancer.
Hemant Roy, MD – University of Nebraska Medical Center; Gastrointestinal Oncology and Hormone Action and Signal Transduction Programs; Understanding the mechanisms by which nonsteroidal anti-inflammatory drugs prevent colorectal cancer.

Alexis Thompson, MD – UCLA Medical School; Pediatric Oncology Program; Bone marrow and stem cell transplantation in pediatric patients.

2003

Hamid Band, MD, PhD – Harvard Medical School; Hormone Action and Signal Transduction Program; Define the role of Cbl-family proteins.

Vimla Band, PhD – Tufts, New England Medical Center; Breast Cancer and Viral Oncogenesis Programs; Delineate the molecular basis of early steps in human breast cancer.

Irina Budunova, MD, PhD – AMC Cancer Research Center; Hormone Action and Signal Transduction in Cancer Program; Mechanisms of skin and prostate tumor genesis.

Serdar Bulun, MD – University of Illinois at Chicago; Breast Cancer and Hormone Action and Signal Transduction in Cancer Programs; Mechanisms of estrogen biosynthesis and regulation of steroidsrogenic genes in human disease.

William Catalona, MD – Washington University School of Medicine; Prostate Cancer Program; PSA-based screening for prostate cancer and the genetics of prostate cancer.

Paul Lindholm, MD – Medical College of Wisconsin; Prostate Cancer Program; Signaling and gene expression of cancer cells that regulate their mobility, invasion and metastasis.

Richard Miller, PhD – University of Chicago; Hormone Action and Signal Transduction Program; Molecular neuroscience and receptor signaling mechanisms.

Elizabeth Perlman, MD – John Hopkins University School of Medicine; Pediatric Oncology Program; Genetic expression analysis of pediatric germ cell tumors.

Gayle Woloschak, PhD – Argonne National Laboratory; Cancer Genes and Molecular Regulation Program; Understanding molecular basis of radiation responses in mammalian cells and in radiosensitive mice.

2004

Edward Grendys, Jr., MD – University of South Florida, School of Medicine; Gynecologic Oncology Program; Clinical research protocols involving ovarian, uterine and cervical cancer.

Mary Hendrix, PhD – University of Iowa; Pediatric Oncology Program; Plasticity of aggressive tumor cells and the epigenetic influence of the microenvironment.

Olke Uhlenbeck, PhD – University of Colorado; Cancer Genes and Molecular Regulation Program; RNA biochemistry.

These individuals are already making important contributions to our research programs. Their presence elevates the image of our Cancer Center and brings us great pride. They serve as mentors to trainees, as role models for junior faculty and as seasoned leaders advancing our scientific agenda.
Notable Cancer Center Member
Leo I. Gordon, MD

When asked how he first became interested in medicine, Leo I. Gordon, MD, credits his family: The son of a physician and a nurse, Dr. Gordon and his twin sister were steeped in all things medical during their Chicago childhoods, so not surprisingly, both grew up to be successful physicians themselves.

Indeed, for Dr. Gordon, the Abby and John Friend Professor of Cancer Research and Professor of Medicine at the Feinberg School of Medicine at Northwestern University; Chief, Division of Hematology/Oncology and Associate Director for Clinical Sciences at the Robert H. Lurie Comprehensive Cancer Center of Northwestern University, medicine and the study of blood diseases and cancer have been a central focus.

After receiving his MD from the University of Cincinnati and completing his internship and residency at the University of Chicago, Dr. Gordon continued studying through fellowships at the University of Minnesota and University of Chicago where, under the guidance of his mentor, the late John Ultmann, MD, he began to concentrate on research, particularly related to lymphomas. "I had a strong attraction to this specialty, because the
science is fascinating and it is readily translated from the laboratory to the patient,” Dr. Gordon says. Now he is a premier clinician-scientist, nationally distinguished for his work in non-Hodgkin’s lymphomas. He joined the faculty of Northwestern in 1979 and became chief of hematology/oncology in 1996.

His current interests lie in radioimmunotherapy of lymphoma and free radical biology. Radioimmunotherapy is a revolutionary treatment using antibodies to deliver radiation to the tumors via I.V. injection, therefore targeting the cancer sites more effectively and sparing the patient the worst side effects linked with traditional radiation and chemotherapy. Free radical biology involves the study of oxygen radicals or reactive oxygen species, which are ubiquitous because they are produced as a consequence of our oxygen-rich environment. It appears that these substances are important not only as possible causes of certain cancers (an observation made by Dr. Sigmund Wietzman, also a member of the Division of Hematology/Oncology and former Division Chief), but paradoxically may be necessary in order to treat certain cancers. The study of our adaptation to oxidant stress is a fundamental theme in Dr. Gordon’s research. Dr. Gordon, along with his colleagues Ron Gartenhaus, MD, Andrew Evens, MD and Sheila Prachand, have been examining cell lines in lymphoma and myeloma in order to study the role of free radicals in cell death pathways. Soon clinical studies of agents which target cell components which generate free radicals and promote death pathways in cancer cells will be started. “We hope that this approach will provide an effective, targeted treatment for lymphoma and other cancers,” says Dr. Gordon.

Dr. Gordon claims time is his greatest foe – and who can argue given the extent of his commitments? In addition to his extensive clinical and laboratory work, journal peer-review and a position on a National Cancer Institute (NCI) study section, Dr. Gordon also has responsibilities as the Chief of the Division of Hematology/Oncology – responsibilities that include managing all faculty matters such as appointments, promotions and salaries, overseeing Northwestern Memorial Hospital’s hematology/oncology inpatient unit, and heading up the hematology/oncology practice group, Northwestern Medical Faculty Foundation. He is as committed to teaching as he is to research: he is responsible for the three-year fellowship training program, which currently has 20 fellows in various levels of training, lectures to sophomore medical school students and trains the hematology/oncology residents. He has more than doubled the size of the hematology/oncology division in the past six years and has built up the program so that it comfortably rests in the top 10 of cancer programs in the country. Dr. Gordon believes that people in leadership positions should strive to create an environment where talented people can succeed.

Dr. Gordon has clear designs on the future of cancer. “Successful treatment of cancer lies in the study of molecular biology and physiology and the ability and foresight to translate that knowledge so that it can be applied to patients. It’s figuring out what makes cells work and what makes them get out of control, and how to reverse that process.” With a wealth of knowledge, skills and acumen, Dr. Gordon is well on his way to achieving his goal.
A native of Greece, Leonidas C. Platanias, MD, PhD, initially became interested in medicine as a child, perhaps the first glimpse of his inventive spirit since no one in his family had a medical background. He was first introduced to oncology early in his studies at the University of Patras Medical School in Patras, Greece, where he earned his MD and PhD. "I learned that so many were affected by cancer, and the idea of helping such a significant number of people made me realize the far-reaching implications of work in this field," says Dr. Platanias, and with this realization began his focused medical aspirations.

At the University of Patras Medical School – a new academic prototype based on the American medical school model – the faculty were medical professionals recently returned from teaching and working in the United States. Dr. Platanias’ professors inspired and encouraged him to continue his career in the U.S. upon graduation, and in 1984 he landed a Fogarty Fellow position at the prestigious National Institute of Health in Bethesda, Maryland. Researching aplastic anemia, he began the immunology studies that would subsequently play a role in his career direction.
After completing his research training at the National Institute of Health, Dr. Platanias began his internal medicine residency in 1986 at State University of New York in Brooklyn, New York. Working in an extremely underprivileged urban area at the beginning of the AIDS epidemic, he “saw a staggering number of AIDS cases” which motivated him to also conduct research on the hematology of the disease, an unusual accomplishment for a resident.

Upon completing his residency and becoming Board Certified in Internal Medicine – the first doctor in his family – Dr. Platanias moved to the Midwest in 1989 to become a fellow in Hematology/Oncology at the University of Chicago. It was during his fellowship that he officially became a clinical oncologist and began to research in the field that would become his career specialty – molecular biology and signal transduction.

Dr. Platanias’ first faculty appointment was in 1992 as Assistant Professor of Medicine at Loyola University in Maywood, Illinois, and here he established his own lab and conducted independent research. Securing a five-year grant from the National Cancer Institute (NCI) to study the role of IRS-proteins in Type IFN signaling and a two-year grant from the Department of Veterans Affairs to study signal transduction of the interferon alfa receptor in neoplastic cells, Dr. Platanias’ research notoriety was on the rise. In 1993, he won the highly recognized American Society of Clinical Oncology, Young Investigator Award and was honored with the prestigious American Cancer Society Career Development Award from 1993 to 1996.

Joining the University of Illinois at Chicago faculty as Associate Professor in 1996, Dr. Platanias was promoted to Professor of Medicine in 2001. He served as Chief of Hematology/Oncology from 2000-2001 and was the Director of the Cellular Signaling Program at the University of Illinois Cancer Center from 2001-2002. It was at the University of Illinois that Dr. Platanias’ expanded research endeavors achieved increased national status and gained significant funding support: a three-year grant (2001-2003) from the American Cancer Society to study signaling pathways mediating hematopoietic stem cell suppression; a five-year grant (2002-2007) from the National Cancer Institute to study signal transduction of Type I interferons in malignant cells; and a four-year grant (2002-2006) from the National Cancer Institute to study the mechanism of action of interferon in chronic myelogenous leukemia.

In May of 2002, Dr. Platanias became the Deputy Director of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University. As an endowed Chair of the Lurie family, Dr. Platanias is a Jesse, Sara, Andrew, Abigail, Benjamin and Elizabeth Lurie Professor of Oncology as well as a Professor of Medicine at the Feinberg School of Medicine, Northwestern University. About his decision to come to Northwestern University, Dr. Platanias shares, “Northwestern is a great university, and the Robert H. Lurie Comprehensive Cancer Center of Northwestern University has become the premier facility of this type in the region, a place of rapid growth and expansion where I can realize both my academic and professional aspirations.”

Dr. Platanias continues his grant-based molecular biology and biochemistry research in signal transduction for cytokines in order to understand the mechanisms within the cancer cells by which various substances affect functionality. As Deputy Director, Dr. Platanias has many oversight responsibilities in addition to his research and teaching. He oversees the Shared Resource Core Facilities, a network of IR facilities that provide various support services for investigators of the Cancer Center. He also directs the development of the Cancer Center’s efforts to procure new, significant research.
grants, especially funding from the National Cancer Institute. Additionally, Dr. Platanias chairs several search committees and supervises the recruitment of new oncology faculty and researchers for the Robert H. Lurie Comprehensive Cancer Center of Northwestern University.

Committed to involvement on a national scale, Dr. Platanias currently participates on several grant review committees: the National Institute of Health CAMP Study Section; the Department of Veterans Affairs Hematology Merit Review Grants Subcommittee, where he now serves as Chairman; and the Leukemia Research Foundation Medical Advisory Board.

When asked about his professional goals, Dr. Platanias emphasizes, “I want to contribute to the Robert H. Lurie Comprehensive Cancer Center of Northwestern University’s continued expansion and success.” A key to that growth, he believes, is the new Robert H. Lurie Medical Research Center of Northwestern University, a $200 million high-tech medical research facility to be completed in early 2005 on the Chicago campus. “The new Medical Research Center will help us recruit high-caliber researchers, build a stronger research structure and facilitate the translation of clinical studies – taking research from the bench to the bedside – which is our ultimate goal.”
Investigating the Possible Effects of Tomatoes or Lycopene in Preventing Prostate Cancer

Peter H. Gann, Susie Lee, Misop Han, Ryan Deaton and Vijayalakshmi Ananthanarayanan

By now, marketing from the food industry has made many Americans aware of research suggesting that lycopene – a compound found in tomatoes – could have protective effects against heart disease and cancer. Prostate cancer is the disease most often implicated in this
research, and as the most commonly diagnosed cancer among men in the U.S., it amounts to an expected 230,110 new cases and 29,900 deaths in 2004. Our group is engaged in conducting a unique randomized clinical trial at Northwestern that is aimed at clarifying the direct effects of tomato on the prostate gland.

The first evidence suggesting a link between tomatoes and prostate cancer risk arose from epidemiological studies reported 8-12 years ago. The most significant was based on the Health Professionals Follow-up Study, a cohort established at Harvard of over 44,000 male health professionals who have regularly recorded their dietary intake, other lifestyle factors and specific health events since 1986. The investigators computed the risk of developing prostate cancer in this cohort as a function of the amount of dietary antioxidants, including lycopene, consumed by the men. The results were essentially negative for all antioxidants (including β-carotene and vitamin E) except lycopene. Risk of developing prostate cancer was 21% lower among men in the highest quintile for lycopene consumption compared to those in the lowest quintile. Further analysis showed that this risk reduction was primarily attributable to men who ate more cooked tomato products, such as tomato sauce. It had been shown previously that cooking tomatoes and eating them with oil substantially increases the bioavailability of lycopene.

The hypothesis was pursued by one of the authors using blood samples available for another Harvard-based cohort: the Physicians’ Health Study. Gann, et al analyzed plasma samples collected and frozen in 1982 from 578 men in this cohort who subsequently developed prostate cancer, and similar plasma samples from 1294 control men who were matched on age and remained free of prostate cancer. This study design, referred to as a nested case-control study, is a powerful way to estimate the association between a biomarker and cancer risk using archived samples collected long before, and hence unaffected by, the disease of interest. The results were strikingly similar to the findings from the analysis of dietary intake – lycopene was the only dietary antioxidant measured in plasma that showed an inverse association with prostate cancer risk. The results were stronger for aggressive (advanced or high-grade) cancer than for all cancers, suggesting a possible late effect on progression. Figure 1 below shows the relative risks for aggressive prostate cancer by quintiles of plasma lycopene concentrations.

Because the Physicians’ Health Study was a randomized trial of β-carotene supplements, the results were stratified to look at the β-carotene and placebo groups separately. The group not receiving β-carotene (placebo group) showed a strong, linear inverse association between lycopene level and risk (P for trend = 0.006). Interestingly, there was no trend for lower risk as lycopene increased in the β-carotene group; however, compared to men in the group with the lowest lycopene level and no antioxidant supplement, the risk for prostate cancer was significantly lower among all men taking β-carotene. One interpretation is that an equivalent antioxidant benefit can be achieved either...
through eating a diet rich in lycopene or taking β-carotene supplements.

Tomatoes are the predominant source of lycopene in most diets, although significant amounts can also be found in watermelon, papaya, guava and pink grapefruit. The chemical structures of lycopene and β-carotene are shown in Figure 2. Lycopene is a 40-carbon carotenoid with 13 double bonds. β-carotene is formed from lycopene by cyclization of its two end groups. Because it lacks β-ionone rings, lycopene cannot be cleaved to form vitamin A, which means more remains available for antioxidant activity. In addition, lycopene’s extra double bonds and unusual stereochemical properties contribute to its potent antioxidant effects. The singlet oxygen quenching ability of lycopene in vitro is at least twice as high as that of β-carotene, and is higher than any other dietary carotenoid studied. These facts, combined with the observation that, in most American populations, lycopene concentrations in serum are higher than any other carotenoid, suggest that the biological importance of lycopene was previously underestimated. Lycopene concentrations in the prostate are particularly high, and are correlated with serum levels.

There is a danger in assuming that pure lycopene supplements are the best type of agent to study, however. Researchers learned from a painful experience with β-carotene, that focusing on a single chemical in foods can sometimes produce unexpected results. Despite decades of research suggesting that men who ate diets rich in β-carotene or had high blood levels experienced a reduced risk for lung cancer, two very expensive Phase 3 randomized trials among male smokers found that supplements actually significantly increased the risk of lung cancer (by about 20%) compared to placebo. More recently, an interesting animal study prompted the same concerns about lycopene. Boileau et al reported, in a well-controlled study using the N-methyl-N-nitrosourea (NMU)-androgen rat carcinogenesis model, that whole tomato powder inhibited the development of prostate cancer compared to a control diet, while a pure synthetic lycopene supplement did not. In the tomato powder group, risk of developing lethal prostate cancer was reduced by 26% compared to controls, versus a non-significant reduction of 9% in the group receiving pure lycopene. In addition to lycopene, other known carotenoids in tomatoes and tomato-based products include β-carotene, γ-carotene, ζ-carotene, phytofluene, and phytoene, all of which are among the 10 major carotenoids that are found to accumulate in human prostate tissue. There are also numerous non-carotenoid compounds in tomatoes that have potentially relevant activity and a large number of unknown phytochemicals as well. Carotenoids generally occur in the plant for a purpose, for example, to protect seeds in fruit from photodegradation and oxidative damage. From an evolutionary perspective, it makes sense that plants would develop sets of interacting compounds to accomplish these functions rather than rely on single compounds.

With all this in mind, we have designed our current trial to evaluate the effects of a tomato extract, which can be delivered in a capsule with a consistent concentration of lycopene. To produce the capsule, specially-bred (non-genetically modified) tomatoes are grown at a facility in Israel (LycoRed Natural Products Industries Ltd.), and water is removed, leaving the complete lipid fraction from the tomatoes,
literally tomato oil. Placebos using a red dye in a lipid vehicle will also be used. Eligible participants will be men with high-grade prostatic intraepithelial neoplasia (HGPIN), a lesion believed to be a precursor to prostate cancer. These men, who will be enrolled from the Urology Clinic at NMH, normally undergo repeated blind biopsy of the prostate. We will obtain prostate biopsy samples before and six months after random assignment to either two capsules per day of tomato extract or placebo. The daily dose, in terms of lycopene, will be 30 mg, which is the approximate level consumed in food by the top decile of U.S. men. The recruitment goal, over 18 months, is a total of 80 patients.

The primary endpoints are specific molecular, histological and nuclear changes in the post-treatment compared to pre-treatment biopsy tissue. We have carefully selected a panel of immunohistochemical markers including proteins that have demonstrated differential expression in the earliest phases of prostate carcinogenesis, in particular during progression from normal to preneoplastic phases. These markers reflect disturbances in proliferation (Ki67, mcm2), differentiation (PSA, chromogranin A), growth factor regulation (EGF receptor, IGF-1), apoptosis (bcl-2, caspase 3) and angiogenesis (CD34 for microvessel density). In addition to standard IHC technique, an important aspect of this project will be the application of computer-based image analysis to provide more accurate, higher throughput quantification of protein expression in prostatic tissue. We will also investigate differential expression of some of the chosen markers in basal versus luminal epithelial cells, because it appears that these cells behave differently during early prostate carcinogenesis. AMACR – α-methylacyl-coenzyme A racemase – has been shown to be strongly upregulated in prostate cancer; expression in HGPIN is variable. We have found some staining for AMACR in normal biopsy tissue, as demonstrated in Figure 3, which shows a section containing both normal glands and cancer. Highly variable staining within the normal areas can be seen within this single section. Recently, we have found that patients who subsequently go on to be diagnosed with prostate cancer have more AMACR staining in their earlier normal biopsies than patients who remain free of prostate cancer. These results will be presented at the Annual Meeting of the American Association for Cancer Research in April, 2004.

We are also working to develop a nuclear morphometry index for the prostate that combines information on nuclear size, shape, DNA content and chromatin texture. To accomplish this, we have collaborated with Bacus Laboratories (Lombard, IL), which has developed a computer-based image analysis system for objective quantification of nuclear grade in other chemoprevention settings.

Briefly, this system takes a digital scan of the tissue under the microscope and using special software extracts nuclei from the image and places them into orderly galleries. Once in galleries, these nuclei are subjected to a large array of measurements involving size, shape, DNA content or texture parameters. Figure 4A shows an example of such a gallery displaying
normal prostate epithelial nuclei with perimeter measurements from a single patient we have studied. Figure 4B shows a graph of the distribution of nuclear grade scores (reflecting the sum of several individual morphometric features) from a patient with high-grade cancer compared to a population of normal nuclei. The mean nuclear grade score is 13.2 standard deviations above the mean for normal nuclei. One can see from the lack of overlap in the curves that this approach provides a powerful way to detect subtle changes in nuclear structure such as those accompanying early neoplastic change.

In conclusion, it is too early to tell whether tomatoes or lycopene have something to tell us about how to prevent prostate cancer. It is our hope, however, that trials like ours, and the new investigational tools we are developing, will give us a sound basis for designing the long-term Phase 3 trials that will be needed to arrive at definitive answers.

Acknowledgements
We wish to acknowledge the important contributions of several other individuals to this work, including Irene Helkowski, MS, Erin Anderson, Ximing Yang, MD, PhD, Robert Meyer, Michael Pins, MD, James W. Bactus, PhD, and Val Kagan, PhD.

Funding
Effort on this project is supported by R01 CA 90759-01A1 and P50 CA90386-02 grants from the National Institute of Health and the National Cancer Institute.

REFERENCES


Four-Dimensional Elastic Light-Scattering Fingerprinting for Early Detection of Colon Carcinogenesis

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Michael J. Goldberg, MD, is the head of the Division of Gastroenterology, Evanston Northwestern Healthcare. He is also an Associate Professor at the Feinberg School of Medicine, Northwestern University.

Ramesh K. Wali, PhD, is a Research Associate Professor in the Department of Medicine at Evanston Northwestern Healthcare/Northwestern University, and a member of the Cancer Center’s Cancer Cell Biology and Gastrointestinal Oncology Programs.

Young L. Kim is currently pursuing his PhD in biomedical engineering from Northwestern University. His research interests include early cancer diagnostic and chemoprevention using light scattering.

Yang Liu is currently pursuing her PhD in biomedical engineering at Northwestern University. Her research focus has been working on the development of light scattering for the early cancer diagnosis.

Colorectal neoplasms are the second leading cause of cancer deaths in the United States, underscoring the public health imperative for developing novel strategies to combat this malignancy1. Screening has been shown to decrease colorectal cancer (CRC) mortality by both identifying lesions at an early, potentially curable stage and also through prevention of...
CRC development by targeting the precursor lesions, the adenomatous polyps\(^2\). However, there are many barriers to widespread implementation of these strategies including patient non-compliance, discomfort, economic constraints, resource availability, and complication risk\(^3\). Indeed, most eligible subjects do not receive any type of CRC screening. Therefore, it is clear that improved screening methodologies are essential to decrease the number of CRC fatalities. Many screening techniques are designed to exploit the “field effect” of colon carcinogenesis, the proposition that the genetic/environmental milieu that results in neoplasia in one region should be detectable throughout the mucosa\(^4\). While several histologic (e.g. rectal aberrant crypt foci (ACF), cellular (e.g. apoptosis in the uninvolved mucosa), molecular (e.g. colonic protein kinase C activity) markers have shown a statistically significant correlation between rectal assays and colonic neoplasia, their performance characteristics are suboptimal for clinical practice. Novel techniques to detect the field effect are, therefore, urgently needed.

There are several lines of evidence that subtle perturbations in colonic micro-architecture may be a manifestation of the “field effect”\(^5\)\(^-\)\(^7\). While micro-architectural alterations may serve as an excellent marker of the “field effect” of colon carcinogenesis, current technology does not allow its practical and accurate detection. Advances in biomedical optics have the potential of enabling real-time \textit{in vivo} assessment of intracellular structure. Light scattering signals are extremely rich and complicated, thus having the potential of yielding unprecedented insights into the micro-architectural organization of the cell. Light scattering signals from intracellular structures depend not only on size, shape, and internal organization, but also on its position as part of a cell itself or a larger organelle (the immediate surrounding milieu of solid particles such as proteins).

In order to realize the full promise of light scattering, we developed four-dimensional elastic light-scattering fingerprinting (4D-ELF), a new generation of optical technology\(^8\)\(^-\)\(^9\). This technology allows us to obtain quantitative information about biological structures without the need for tissue biopsy, fixation, staining, or other processing. 4D-ELF enables probing tissue organization at scales from tens of nanometers to microns, thus encompassing a spectrum of structures ranging from macromolecular complexes to whole cells. Indeed, this provides information about objects 20-50 times smaller than can be detected by conventional microscopy. Thus, the light scattering fingerprints provide a heretofore unattainable insight into the architecture of living tissue at the nanoscale organizational level. The data obtained from light scattering fingerprinting should not be considered as a mere substitution for the morphological tissue analysis using light microscopy. The four-dimensional information extracted from ELF provides much greater biological insights than the previously utilized technologies. The critical advantages are related to the quantitative information regarding nano-scale architecture on living tissues. 4D-ELF gives information at the level of electron microscopy and yet keeps the levels of cellular organization that may be lost with staining/fxation allowing hereto undiscovered insights regarding micro-architectural changes that occurs early in neoplastic transformation.

Our objective of this study was to assess whether 4D-ELF would be able to detect the field effect of colon carcinogenesis. In the present studies, we tested 4D-ELF in the azoxymethane (AOM)-treated rat, a well-validated model of colorectal carcinogenesis that recapitulates many of the important morphological, genetic and cellular alterations seen in human colon cancer\(^10\). We demonstrate that 4D-ELF is able to accurately identify alterations in the colonic mucosa at a far earlier stage than any previously described markers. Furthermore,
these changes correlated well with the carcinogenic progression in this model.

**Methods**

*Light Scattering Fingerprinting.* We developed a specialized light scattering instrument to measure comprehensive light scattering data from living tissues. Briefly, a series of points on a sample surface were illuminated by a collimated linearly polarized light from a Xe-lamp. A Fourier lens positioned in the collection arm of the instrument projected the angular distribution of the backscattered light onto the slit of a spectrometer, which further diverted this light now in the direction perpendicular to the slit according to its spectral composition. The resulting 2D image, wavelength $\lambda$ (400-700 nm) vs. scattering angle $\theta$ (0°-7°), was projected onto a CCD (Roper Scientific) for a given azimuth of scattering $\phi$, which was varied by rotating a polarizer in the delivery arm of the system. The instrument also measures two independent polarization components $p$ of the scattered light: one polarized along, $I_{||}$, and orthogonally, $I_{\perp}$, to the incident polarization. Such 4D data ($\lambda, \theta, \phi, p$) provide comprehensive information about the light scattering and can serve as extremely sensitive “fingerprints” of the specimen microarchitecture. Furthermore, the differential polarization signal $\Delta I(\lambda) = I_{||}(\lambda) - I_{\perp}(\lambda)$ is particularly sensitive to the superficial tissue (<50µm), e.g. epithelium. This is critical for early detection of pancreatic precancer as it is of epithelial origin. Signals $I_{||}, I_{||} + I_{\perp}, \text{ and } I_{\perp}$ contain information about progressively deeper tissues.

In order to analyze the light scattering signatures, we assayed a variety of parameters that span the spectrum of micro-architectural abnormalities. The spectral slope analysis evaluates size distribution of particles ranging from macromolecules to organelles. Fractal dimension, on the other hand, reflects alterations of the tissue organization at much larger scales, ranging from large organelle to groups of cells.

Principle component analysis (PCA) is a standard data procedure for assessing underlying structure in a data set. In order to infer relationship to colon carcinogenesis, we correlated the 4D-ELF signatures with the subsequent occurrence of ACF. Specifically, neoplastic signatures should progress over time and be predominantly in the distal colon especially early during carcinogenesis (mirroring our ACF data). All data from AOM-related signatures were compared with an age matched saline-treated rat.

**Animals.** All animal studies were performed in accordance with the institutional animal care and use committee of Evanston-Northwestern Healthcare Forty-eight (48) male Fisher 344 rats (150-200 g) were randomized equally to groups that received either two weekly i.p. injections of AOM (15 mg/kg) (Sigma, St Louis Mo) or saline. Rats were fed standard chow and were sacrificed at various time points after second injection (2,4,5,6,8,12 and 20 weeks). Colonies were removed, flushed with phosphate buffered saline and divided into equal proximal and distal segments. 4D-ELF analysis was performed on fresh tissue. ACF quantitation was performed on a subset of animals using methods previously described.

**Results**

The AOM-treated rat model is one of the most robust and widely used models of colon cancer. As in human carcinogenesis, in this animal model, neoplasia progresses through a well defined sequence of events. In AOM-treated rats, the earliest detectable markers of carcinogenesis, ACF, develop in 5-12 weeks after the AOM injection; adenomas are observed in 20-30 weeks, and carcinomas develop after more than 40 weeks. No histologic, molecular or genetic markers have been shown to be able to detect earlier stages (<4-12 weeks) of colon carcinogenesis. Here we report that light scattering fingerprints change profoundly as early as 2 weeks after AOM-injection.
We recorded ELF data from AOM-treated and control rats at 2, 4, 5, 6, 8, 12, and 20 weeks after AOM or saline injection. In order to correlate ELF with a well-validated biomarker of colon carcinogenesis, we analyzed ACF number on a subset of animals in this study. ACF were detectable at week 4 and progressively increased in both number and complexity over the course of the experiment (data not shown). There was a marked distal predominance in ACF. While proximal ACF did occur, these required longer to develop and were less numerous than distal ACF. There were no ACF detected in the saline-treated animals.

Figure 1 shows representative light scattering fingerprints recorded from rat colons 2 weeks post-AOM treatment or age-matched control animals. As evident from Fig. 1(a) and (b), in the proximal colon, where the carcinogenic effect of AOM is minimal, AOM-treatment induces only modest changes in ELF. For comparison, in the distal colon, the alterations of the fingerprints are dramatic (Figs. 1(d) and (c)), paralleling the carcinogenic efficacy of AOM in the distal colon. We note that the time point, for which the alteration of light scattering fingerprints was detected (i.e. 2 weeks after AOM-treatment), preceded the formation of ACF or any other currently known markers of colon carcinogenesis.

As outlined in the Methods, we identified several light scattering markers that can be obtained from the ELF data and are highly significant for the earliest precancerous changes in the colons of AOM-treated rats. These alterations in light scattering markers indicate that the nano/micro-architecture of tissue changes even in the earliest stages of colon carcinogenesis. Importantly, the changes in light scattering markers follow both the spatial and temporal progression of colon carcinogenesis (Table 1 and Fig. 2). Moreover, the performance characteristics of light scattering markers

<table>
<thead>
<tr>
<th>Light Scattering Marker</th>
<th>P-value (2-20 weeks post AOM-treatment)</th>
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<tbody>
<tr>
<td>Spectral slope</td>
<td>&lt;10^-14</td>
</tr>
<tr>
<td>PC 1</td>
<td>&lt;10^-42</td>
</tr>
<tr>
<td>Df</td>
<td>&lt;10^-9</td>
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Table 1: Significance of light scattering markers of early colon carcinogenesis measured using ANOVA. Spectral slope was measured as the absolute value of the linear coefficient of the linear fit to $\Delta I(\lambda)$ and characterizes the size distribution of microscale tissue structures. PC1 – the first principal component obtained using principal component analysis of $\Delta I(\lambda)$. PC1 accounted for >90% of the data variance. Df – fractal dimension of tissue microarchitecture measured as the linear slope of two-point mass density correlation function $C(r)$ in the linear region of log-log scale. ($C(r)=\langle \rho(r)\rho(r'+r)\rangle$, where $\rho(r)$ is a local mass density at point r with 1 $\mu$m<r<50 $\mu$m.)

Table 2: Performance characteristics of light scattering-based diagnosis using the markers listed in Table 1. 2 weeks post AOM-treatment: neoplastic changes cannot be detected using histologic or molecular means; 12 weeks post AOM-treatment: the earliest time point when neoplastic changes can be detected histologically, although with low sensitivity and specificity.
dramatically exceeded all other conventional and experimental markers for all time points (Table 2).

Discussion

Exploitation of the “field effect” in colon carcinogenesis is a common theme in colon cancer screening. As previously discussed, present strategies lack sufficient sensitivity and specificity for optimal population screening. Thus, the finding of an accurate marker for the field effect would be of major clinical importance. Application of the newly developed 4D-ELF technology has great promise for colon cancer screening because of the remarkable sensitivity to the earliest changes in carcinogenesis. Utilizing quantitative analysis of tissue micro-architecture, we were able to detect the earliest alterations in neoplastic transformation (at 2 weeks after carcinogen treatment) which may reflect the “field effect”. The relevance of these 4D-ELF changes to carcinogenesis is supported by both the temporal and spatial correlation. Temporally, the marked alterations detected at week 2 progressively increased in magnitude over time consonant with the neoplastic effects of AOM in this model. Spatially, the early signature alterations were predominantly in the distal colon, the region of the colon most susceptible to ACF and tumor development. Moreover, the changes noted with 4D-ELF occurred at 2 weeks after AOM-treatment, a time-point far earlier than seen with other conventional biomarkers. This time-point was of particular importance in that the nonspecific genetic and cellular changes associated with acute effects of carcinogen have dissipated. Therefore, alterations at this time reflect the earliest changes related to the field effect of carcinogenesis. The biological mechanisms of this phenomenon are currently under investigation. We believe that our data provide compelling evidence that the micro-architectural perturbations in the histologically normal mucosa identified by 4D-ELF represent a reliable marker of the “field effect” of colon carcinogenesis. Moreover, it is important to note that despite the extensive “data-mining” performed on 4D-ELF signatures, this represents less than 5% of total information available. Therefore, it is conceivable that our findings of early changes in carcinogenesis may be eclipsed by future ELF analyses.

Figure 2. Analysis of 4D-ELF information. (a): Changes in the spectral slope at 2, 4, 5, 6, 12 and 20 weeks after the AOM treatment in the distal colons of control and AOM-treated rats obtained by means of the analysis of the spectral dimension in ELF. (b): Changes in the fractal dimension of the superficial mucosa obtained by means of the analysis of the angular dimension in ELF. (c): Principal component analysis (PCA) of light scattering fingerprints: score of Principal Component 1 (PC1). The changes in all three ELF markers are significant even for the pre-ACF time point (2 weeks post AOM treatment). Moreover, in agreement with the ACF data, the change in the ELF markers was even more dramatic at the later time points.
In summary, this report demonstrates that the newly developed technology, 4D-ELF was able to detect heretofore unrecognized subtle micro-architectural perturbations from the field effect of colon carcinogenesis. This technology has promise of allowing accurate risk stratification and identifying patients who would benefit from colonoscopic screening. One can envision the rapid “bench-to-bedside” transition of this technology through the development of an endoscopically compatible probe with real-time signature determination. Moreover, 4D-ELF can give unparalleled insights into biological changes early in carcinogenesis. Further studies are being conducted to translate this technology into clinical practice and to determine the biological determinants of these micro-architectural alterations.

This study was supported in part by research grants from the National Institutes of Health (1R21CA102750-01), National Science Foundation (BES-0238903), General Motors Cancer Research Foundation, and American Cancer Society-Illinois Division.

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Epstein-Barr virus (EBV) is a gamma-herpesvirus that infects B-cells and epithelial cells. Epidemiological studies indicate that a latent EBV infection is associated with proliferative disorders of lymphoid and epithelial cells including infectious mononucleosis, endemic Burkitt's lymphoma, and nasopharyngeal carcinoma. EBV is latent in these proliferating cells, so that very few viral genes are expressed, and no infectious virus is released. EBV's genome is present as a nuclear plasmid within latently infected cells. This plasmid is replicated once per cell-cycle in synchrony with cellular chromosomes. Further, in approximately 97% of mitotic events, newly replicated viral genomes are partitioned equally into daughter nuclei. This efficiency equals the efficiency observed for the partitioning of plasmids containing chromosomal centromeres in yeast, indicating that the partitioning of EBV genomes is an equally efficient process. Once per cell-cycle replication of viral genomes, and their subsequent equal partitioning require a single viral protein, the Epstein-Barr nuclear antigen 1 (EBNA1). EBNA1 binds a region of the viral genome termed the origin of plasmid replication (oriP). Consistent with this central role for EBNA1 in a latent EBV infection, it is the only EBV protein...
that is expressed in all malignancies associated with EBV. OriP has two clusters of binding sites for EBNA1, termed the dyad symmetry element (DS), and the family of repeats (FR)\[^{11}\]. EBNA1 bound to DS recruits the cellular origin recognition complex (ORC), and the cell-cycle dependent MCM complex to DS, to initiate DNA synthesis from oriP\[^{12,13}\]. EBNA1 bound to FR is required to maintain EBV’s genome in proliferating cells, and to partition genomes into daughter cells\[^{14}\]. Consistent with this, deletion of FR from EBV eliminates the ability of the virus to immortalize cells and establish latency\[^{15}\].

**Domains of EBNA1**

We study how EBNA1 bound to FR mediates the maintenance and partitioning of viral genomes in proliferating cells. For this, we make use of small plasmids that contain oriP introduced into human cell-lines that express wild-type EBNA1, or derivatives thereof. These small oriP-plasmids are more readily manipulated than the large viral genome, but retain the properties of being replicated once per cell cycle and then partitioned equally\[^{14,16,17}\]. EBNA1 is schematically represented in Figure 1. The C-terminal one-third of the protein (a.a. 451-641) dimerizes and then specifically associates with EBNA1-binding sites within the DS and FR regions of oriP\[^{2}\]. The central one-third of the protein (a.a. 90-327) contains a repeat of glycine-glycine-alanine. Most of this region can be deleted without affecting EBNA1’s functions in replication, maintenance or partitioning in the context of human cell lines in vitro\[^{10,18}\]. The gly-gly-ala repeats are flanked on either side by positively regions that we refer to as “A” (a.a 33-89) and “B” (a.a 328-378). Previous work had demonstrated that chimeras of either of these regions fused to GFP associated with metaphase chromosomes\[^{20,21}\]. Region B also associates with a nucleolar protein, termed EBP2, and it has been hypothesized that this interaction mediates EBNA1’s ability to partition EBV genomes\[^{16,18}\].

**EBNA1 is an AT-hook protein**

In addition to being positively charged, both region A and region B share a second characteristic. They contain alternating glycine and arginine residues, as indicated in Figure 1. Such repeats are found in cellular proteins that bind metaphase chromosomes through a DNA binding motif termed the “AT-hook”. In an AT-hook, the alternating glycine and arginine residues form a flexible interface that can closely approach the minor groove of DNA. Within the minor groove, there is a specific interaction between the guanadino group of arginine and O6 atom of thymidine\[^{22}\]. This renders an AT-hook specific for AT-rich DNA. Because EBNA1 had sequences resembling cellular AT-hooks, we tested the hypothesis that domains A and B of EBNA1 interacted with cellular chromosomes by functioning as AT-hooks.

For this, we examined whether baculovirus-expressed EBNA1 would specifically associate with AT-rich DNA through the use of a nitrocellulose filter-binding assay. Our assays indicated that the binding
of EBNA1, or the cellular AT-hook protein, HMGA1a, to a labeled poly(dA.dT) probe was readily competed by cold poly(dA.dT), but not by other nucleic acids such as phage lambda DNA, poly(dG.dC) or even oriP (Figure 2A). Additional assays performed with purified peptides corresponding to domains A and B confirmed that the AT-hook activity resided in each of these domains. To confirm that the binding was in the minor groove, we tested whether association of domains A and B with the labeled poly(dA.dT) probe could be competed by the minor groove binding drug distamycin (Figure 2B), as it is known to compete the binding of HMGA1a from such a probe. These studies indicated that distamycin A competed domains A and B from binding the labeled poly(dA.dT) probe, providing evidence that domain A and domain B bind poly(dA.dT) in the minor groove, just as HMGA1a does.

**AT-hooks are required for the licensed replication of EBV**

Although both domains A & B are AT-hooks, EBNA1’s ability to stably replicate and partition oriP plasmids has been postulated to occur through the interactions of domain B with EBP2. To test whether this interaction was necessary for EBNA1 to function, we constructed three derivatives of EBNA1 that lacked domain B. In the first two, we deleted domain B, and replaced it with either one additional copy of domain A or two additional copies of domain A. These derivatives are called 2A-DBD and 3A-DBD. In the third derivative, we deleted the entire amino-terminus of EBNA1, and replaced it with the cellular AT-hook protein, HMGA1a. This derivative is called HMGA1a-DBD. We tested whether 2A-DBD and 3A-DBD, that lacked the ability to associate with EB2 could still bind metaphase chromosomes. A representative analysis with 2A-DBD is shown in Figure 3A, where the EBNA1 derivative can be observed as bright speckles on Hoechst-stained metaphase chromosomes. Metaphase chromosomes were isolated from 293 cells stably expressing 2A-DBD stalled with colcemid. Indirect immunofluorescence was performed using the K67.3 rabbit polyclonal antibody against the DBD of EBNA1. Individual layer images (Z-sections of 100 nm) were captured at 100X and deconvolved. 2A-DBD, as well as 3A-DBD (not shown) were observed to localize to metaphase chromosomes in discrete punctate spots that resemble those observed with wild-type EBNA1.

**Figure 2.** EBNA1 supports specific affinity for AT-rich DNA in vitro. Purified peptides domain A or domain B of EBNA1 and full-length EBNA1 were analyzed for their ability to bind AT-rich, labeled oligonucleotides compared to full-length HMGA1a in filter binding assays. (A) The association of baculovirus-expressed EBNA1 and HMGA1a with a poly(dA.dT) probe is specific, and competed effectively by cold poly(dA.dT). In contrast, non-specific competitors such as poly(dG.dC) and a HindIII digest of phage lambda DNA do not compete for the binding of these proteins with poly(dA.dT). (B) The AT-hook synthetic analog, distamycin A, specifically competes with the ability of domain A, domain B, and HMGA1a to bind poly(dA.dT) probe. All binding assays were performed using with protocols described by the groups of Hurwitz and Kelly.

**Figure 3.** Versions of EBNA1 that bind DNA directly and lack EB2 binding function like wtEBNA1. (A) 2A-DBD binds metaphase chromosomes. Metaphase chromosomes were isolated from 293 cells stably expressing 2A-DBD stalled with colcemid. Indirect immunofluorescence was performed using the K67.3 rabbit polyclonal antibody against the DBD of EBNA1. Individual layer images (Z-sections of 100 nm) were captured at 100X and deconvolved. 2A-DBD, as well as 3A-DBD (not shown) were observed to localize to metaphase chromosomes. Metaphase chromosomes were isolated from 293 cells stably expressing 2A-DBD stalled with colcemid. Indirect immunofluorescence was performed using the K67.3 rabbit polyclonal antibody against the DBD of EBNA1. Individual layer images (Z-sections of 100 nm) were captured at 100X and deconvolved. 2A-DBD, as well as 3A-DBD (not shown) were observed to localize to metaphase chromosomes. Metaphase chromosomes were isolated from 293 cells stably expressing 2A-DBD stalled with colcemid. Indirect immunofluorescence was performed using the K67.3 rabbit polyclonal antibody against the DBD of EBNA1. Individual layer images (Z-sections of 100 nm) were captured at 100X and deconvolved. 2A-DBD, as well as 3A-DBD (not shown) were observed to localize to metaphase chromosomes. Metaphase chromosomes were isolated from 293 cells stably expressing 2A-DBD stalled with colcemid. Indirect immunofluorescence was performed using the K67.3 rabbit polyclonal antibody against the DBD of EBNA1. Individual layer images (Z-sections of 100 nm) were captured at 100X and deconvolved. 2A-DBD, as well as 3A-DBD (not shown) were observed to localize to metaphase chromosomes. Metaphase chromosomes were isolated from 293 cells stably expressing 2A-DBD stalled with colcemid. Indirect immunofluorescence was performed using the K67.3 rabbit polyclonal antibody against the DBD of EBNA1. Individual layer images (Z-sections of 100 nm) were captured at 100X and deconvolved.
This analysis is shown in Figure 3B. For this, an oriP-plasmid was transfected into cells expressing wild-type EBNA1, 2A-DBD or 3A-DBD. Plasmids were recovered either seven days or twenty-one days post-transfection, and the amount of replicated (DpnI-resistant) DNA was quantified by Southern blot. As shown in the Figure, both 2A-DBD and 3A-DBD permitted the replication of an oriP-plasmid at levels comparable to wild-type EBNA1.

These studies indicate that versions of EBNA1 that contain an AT-hook are sufficient to mediate licensed oriP replication. In previous studies we have demonstrated that oriP-plasmids have to be tethered to metaphase chromosomes for them to be replicated in the ensuing S-phase24. Others have shown that the oriP-binding domain of EBNA1 is not sufficient by itself to support the replication of oriP-plasmids25,26. One model that reconciles these varied observations relies on recent observations that like EBNA1, the ORC complex also associates with AT-rich DNA27,28. Thus we believe it likely that an interaction between EBNA1 and ORC is first mediated by their common localization to AT-rich DNA, on metaphase chromosomes, permitting the subsequent replication of oriP-plasmids in S-phase.

This model explains why the entire amino-terminus (a.a. 1-450) of EBNA1 can be replaced by any protein that binds AT-rich DNA (see below), and yet leave EBNA1 competent to support oriP-replication. On the other hand, deletion of this region creates a derivative of EBNA1 that lacks AT-hooks and is incapable of supporting oriP-replication.

**AT-hooks are required for the partitioning of EBV genomes**

A model for how EBNA1 might partition EBV genomes Studies from a number of groups have shown that eukaryotic chromosomes are not uniformly condensed. They contain highly condensed Q-bands interspersed with less condensed R-bands that are AT-rich. These AT-rich R-bands have been termed as scaffold attached regions (SARs)29-35. HMGA1a, and other AT-hook proteins have been localized to these regions32,36-38, and when visualized on metaphase chromosomes appear as a series of punctate dots (Figure 4A). While we have no direct evidence that EBNA1 associates with SARs on metaphase chromosomes as HMGA1a
does, the distribution of EBNA1 and HMGA1a-DBD on metaphase chromosomes cannot be distinguished, and the maintenance of oriP plasmids by these two proteins is mathematically identical. If EBNA1 were to localize to a SAR on an interphase chromosome, it is possible that after S-phase, EBNA1 along with the replicated daughter oriP plasmid is distributed to the sister SAR on the sister chromatid. The indirect immunofluorescence analysis of some of our metaphase spreads indicates that the distribution of EBNA1 “dots” on some sister chromatids is approximately equal, an observation that is also true for HMGA1a-DBD (See Figure 4A). In this model that is presented schematically in Figure 4B, the per-replicon partitioning event is simply the distribution of EBNA1 to the sister SAR on the newly-replicated sister chromatin. Replication of chromosomal DNA may be sufficient to very transiently displace EBNA1 bound to a SAR and permit reassociation with SARs on both sister chromatids.

**AT-hooks are not sufficient for EBNA1 to activate transcription**

In addition to mediating the replication and partitioning of EBV genomes, EBNA1 activates transcription from at least three EBV promoters. The mechanism by which EBNA1 activates transcription is not clear. It has been reported that EBNA1 activates transcription from episomal reporter plasmids by facilitating their retention within transfected nuclei. If this were the only mechanism by which EBNA1 activates transcription, we would expect that HMGA1a-DBD and wild-type EBNA1 would activate transcription to equivalent degrees as both proteins support plasmid maintenance to similar levels. However, another region of EBNA1 (a.a. 65-89) has been mapped recently outside of the AT-hook regions as a possible transcription activation domain. To determine whether the sole mechanism by which EBNA1 activated transcription was via plasmid retention, we tested the ability of EBNA1, HMGA1a-DBD, or just the DBD to activate transcription from an integrated EBNA1-dependent transcription reporter in BJAB cells. Our results indicate that EBNA1 can transcription from this reporter, while HMGA1a-DBD does not activate transcription to any level above the DBD alone (Figure 5). Thus while the AT-hooks of HMGA1 can substitute for the amino-terminus of EBNA1 to support the replication and partitioning of EBV plasmids, they are insufficient to support transcription activation to the same level as EBNA1.

Cellular AT-hook proteins function in number of processes including replication, chromatin remodeling, recombination, and enhanceosome formation. Some of them are oncogenic. Our future studies will investigate whether EBNA1’s AT-hooks allow it to substitute for a cellular AT-hook protein in any of these processes.
REFERENCES


Filopodia Formation and Cancer Metastasis

Gary Borisy and Danijela Vignjevic

Most anticancer therapeutic drugs target aspects of cell proliferation and have deleterious side effects on rapidly renewing cell populations such as those of the hematopoietic lineage or intestinal lining. In comparison to antiproliferatives, little success has yet been attained in developing therapeutics effective against metastasis. Yet, secondary metastases in vital organs are often the cause of mortality. Metastatic cancer cells have the capacity to escape from a primary tumor, invade the surrounding tissue, cross the endothelial wall of capillaries, become carried through the circulatory system to distant locations, re-cross the endothelial wall and establish secondary tumors in previously unaffected tissues. Identifying the triggers for metastasis and understanding the individual steps of the process have been difficult due to its complex and multifaceted nature. One hallmark of many cancer cells that is thought to be critical for their acquisition of an invasive phenotype is the abundant expression of exploratory, sensory organelles known as filopodia. An understanding of the mechanism of filopodia formation holds the promise of identifying molecular targets for the development of novel anti-cancer therapeutic drugs.
Filopodial function

Filopodia are thin, spike-like protrusions of the cell surface that have at their structural core a bundle of 15-20 actin filaments cross-linked into a stiff array. The tip of a filopodium contains a complex of molecules specialized for signaling and adhesion as well as for organizing the bundle of actin filaments. The actin filaments themselves are all oriented in parallel with their so-called barbed (fast growing) ends toward the filopodial tip. The main cross-linker of the actin filaments is a protein known as fascin.

Most cell types use filopodia as sensing organs to explore the extracellular matrix (ECM) and surface of other cells. In the growth cones of migrating axons, filopodia sample the local environment and efficiently scan a wide terrain as sensory antennae, searching for guidance cues that allow the growing axon to navigate over long distances and find its appropriate target.

Filopodia have a role in cell adhesion and cell spreading. Many morphogenetic events in embryonic development require two free epithelial edges to fuse together and create a continuous epithelium. This fusion process is accomplished by filopodia extending from adjacent epithelial cells and interdigitating with each other. Filopodia also appear to serve as locomotory organelles; e.g. in fibroblasts grown in a three-dimensional matrix of collagen fibers and cells that wander through fluid filled spaces in the body, such as neutrophils. Cancer cells become metastatic by acquiring a motile and invasive phenotype. Recent evidence suggests that this step requires the remodeling of the actin cytoskeleton and the expression of abundant filopodia.

How are filopodia built?

We studied the mechanism of filopodia formation in two model systems: in vitro using cytoplasmic extracts, and in vivo using B16F1 melanoma cells (Fig. 1). In the vitro system, filopodial-like bundles of actin filaments are induced to form on plastic beads. The beads are coated with activators of the Arp2/3 complex which initially nucleates the formation of a branched or dendritic network of actin filaments. Under suitable conditions in vitro, the ultimate result is what we have called “stars”: actin bundles radiating from the bead. Actin filaments in these bundles, like those in filopodia, are long, unbranched, aligned, uniformly polar, grow at the barbed end and have a dendritic network at their roots. Our kinetic and structural investigation of filopodial initiation demonstrated that these filopodial bundles were formed by gradual reorganization of the dendritic network in a process that we have termed the convergent elongation mechanism and that involves elongation of a subset of dendritic filaments, self-segregation of these filaments into filopodial precursors, and initiation of bundling at the tips of the precursors (Fig. 2). This mechanism recognizes three necessary processes for filopodia formation: nucleation, elongation and bundling.

Nucleation

The Arp2/3 complex is thought to play a role in filopodia formation because one of its activators, N-WASP, induces filopodia in cells. However, since Arp2/3 is absent from established filopodia, one may infer that it likely participates in initiation, not in steady state elongation of filopodia. In our in vitro system, formation of filopodia-like bundles indeed depended on the presence and activity of the Arp2/3 complex. Our data suggested that...
The formation of filopodia involves the synthesis of new actin filaments, the addition of subunits at the barbed ends, and the bundling of these filaments to form a bundle.
besides fascin, localized significantly to filopo-dia. Although the contribution of an as yet unidentified cross-linker cannot be excluded, the results of the molecular marker analysis are consistent with the conclusion that fascin is the major bundling protein in filopodia of melanoma cells.

**Fascin – major bundler in filopodia**

Fascin was discovered by Kane et al. in the 1970s as a 55 kDa protein\(^1\). It was named fascin because of its ability to form tight and stable unipolar bundles with F-actin (from Latin, *fasiculus*, a bundle). Since fascin is a monomeric globular protein\(^1\) it must have two actin binding sites. One putative actin binding site is identified with residues 29-42, a highly conserved region in all fascins\(^1\). Another site has been deduced to lie at the C-terminus of the molecule\(^2\).

The first cloned human fascin (fascin 1) is highly similar in vertebrates and invertebrates\(^1\). It is expressed in many vertebrate tissues with particularly high expression in brain. Fascin 1 is not uniformly expressed in all cell types. It appears to be low or absent in epithelial cell lines, but is expressed at high levels by neurons, glial cells, dendritic cells and many epithelial tumor cells\(^1\). Transformed cells express 5-12 times more fascin than the level observed in normal cells\(^2\). Dramatic increases in fascin expression have been noted in many cancers, lymphocytic disorders and hyperplasias. Correlation between fascin expression and tumor stage has been reported in many cancers; e.g. pancreatic ductal adenocarcinomas\(^2\), large-cell neuroendocrine carcinomas\(^2\), large-cell neuroendocrine carcinomas\(^2\), follicular dendritic cell tumor\(^2\), skin neoplasia\(^2\), ovarian cancer\(^2\) and breast cancers\(^2\). Specifically, loss of hormone receptor status in breast carcinomas is associated with increased tumor cell motility and invasiveness. In an immunohistological study of breast cancers estrogen receptor level was inversely correlated with the expression of fascin. Thus, the up-regulation of fascin in hormone receptor-negative breast cancers may contribute to their more aggressive behavior\(^2\). Further, the over-expression of c-erbB-2/ HER-2, a receptor tyrosine kinase, correlates with poor prognosis in patients with breast and ovarian cancer. It has been determined that over-expression of c-erbB-2 is associated with dramatic increases in mRNA and protein levels of fascin\(^2\). Finally, fascin expression is used as a diagnostic marker for particular forms of cancer\(^2\). Examples are: Reed-Sternberg cells as a long-recognized hallmark of Hodgkin’s lymphoma, interdigitating dendritic cell sarcomas and Epstein B virus-transformed B cells.

Although, fascin is localized in filopodia, no functional test had been performed as to whether it is required for their formation. We used targeted depletion by RNA interference as a direct way to investigate the role of fascin in filopodia formation. We prepared a hairpin siRNA expression vector with a GFP marker, pG-Super\(^2\) which is based on the pSuper vector\(^3\) and modified to express EGFP under a separate promoter for the added advantage of easier detection and sorting of expressing cells. The selected sequences did not have significant similarity to any other known genes in the mouse database as determined by BLAST search (NCBI). Thus, these hairpin siRNA constructs were designed to silence fascin without affecting other targets. Two approaches were used to control for the specificity of silencing: expression of siRNAs with mismatching nucleotides and rescue of the knockdown phenotype by expressing a fascin gene that was refractory to silencing. Fascin silencing in mouse B16F1 melanoma cells at the protein level was assayed by immunoblotting and immunostaining. The phenotype of fascin-depleted cells was analyzed by light and electron microscopy. Fluorescence microscopy after phalloidin staining (Fig. 3A) showed that 4 days of expression of fascin siRNA caused significant decrease in the number of filopodia in cells,
whereas their lamellipodia looked unaffected. To quantify the extent of filopodia inhibition, we determined the number of filopodia per unit length of the cell leading edge and found a 4-5 fold decrease in filopodia in fascin-depleted compared to control cells (Fig. 3B). The few remaining filopodia were wavy and loosely bundled as determined by electron microscopy.

Specific recruitment of fascin to filopodia suggests a regulatory mechanism for fascin targeting. Previous works have shown that PKCα-driven phosphorylation of fascin at serine 39, inhibits fascin binding to F-actin in vitro. To examine the relationship between the serine 39 phosphorylation and the filopodia formation, we introduced point mutations into the fascin sequence to mimic the dephosphorylated (S39A) or phosphorylated states (S39E). We expressed these mutants as GFP-tagged proteins in melanoma cells and analyzed the efficiency of filopodia formation, structural organization of filopodia and kinetics of different fascin mutants in expressing cells. Expression of S39A or S39E fascin mutants produced differing phenotypes in terms of length and frequency of filopodia. The S39A mutant, which mimics the active state, induced overabundant filopodia extending laterally from cell edges as well as from the dorsal surface. The total length of filopodial bundles including their internal and protruding parts was also increased after expression of the S39A mutant, and this increase was entirely due to their protruding parts. In contrast, expression of the S39E mutant, which mimics the inactive state, resulted in an approximately 2.5-fold reduction in the number of filopodia. In addition to opposite effects on filopodia formation, we found striking differences in the distribution of two fascin mutants in filopodial bundles (Fig. 3C).

Fluorescence intensity profiles of GFP-fascin and phalloidin-stained actin in filopodia showed that distributions of wild type fascin and active S39A mutant paralleled that of actin, although there were some differences in the detailed shape of the profiles (Fig. 3D). Such distribution is consistent with an idea that these fascins are targeted to filopodia primarily through their interaction with actin. In contrast, inactive S39E mutant was highly enriched at filopodial tips and its fluorescence intensity rapidly declined proximally away from the tip, which sharply contrasted the more flat distribution of actin. Therefore it is plausible to assume a second mode of inactive fascin binding in filopodia that is not directly dependent on actin. In conclusion, by several complementary functional approaches we demonstrated that fascin is the major cross-linking protein in filopodia, which plays a critical role in their formation and protrusion by bundling filopodial actin filaments and providing them with stiffness necessary for pushing.

The role of fascin in cell migration has been investigated by direct perturbation of the actin-fascin interaction using antibodies reactive with the actin-binding sites of fascin, which inhibit binding of fascin to actin. Introduction of these

Figure 3. Effect of fascin depletion and fascin mutant expression on filopodia number in mouse melanoma cells. (A) Distribution of actin revealed by phalloidin staining. Asterisk labeled filopodia is enlarged below. (B) Number of filopodia per 20 µm of cell perimeter. (C) GFP-fascin distribution on filopodia. (D) Relative fluorescent intensity vs distance from the tip of filopodia. Grey is actin and black is fascin.
antibodies into cells completely prevented cell spreading and migration on TSP-1 and also partially blocked cell migration on fibronectin. However, in a different study, it was reported that although fascin-overexpression correlated with the formation of dynamic cell protrusions, the presence of these finger-like protrusions did not show a clear correlation with increased locomotion for cell colonies on planar substrata. On the contrary, overexpression of fascin was reported to significantly increase the migration activity of epithelial cells in three-dimensional, trans-filter assays. Similarly, melanoma cells transfected with fascin exhibited increased migration compared with untransfected cells, an effect that was augmented by addition of NGF. Furthermore, the directed migration of melanoma cells towards NGF was inhibited by expression of mutated fascin S39D. Thus, fascin by participating in the formation of cell protrusions may promote cell migration in vitro in three-dimensional matrices. We propose that fascin may promote metastasis of cancer cells by participating in the formation of filopodia. However, overexpression of other molecules involved in filopodia formation could also facilitate metastasis. An example could be ezrin, a cytoskeletal linker between the plasma membrane and actin filaments which has been found to be associated with metastasis of certain cancers.

Conclusion
Cancer metastasis is a significant problem and a tremendous challenge to drug discovery relative to identifying key therapeutic targets as well as developing breakthrough medicines. We propose that key molecules involved in filopodia formation such as fascin and its regulatory elements could serve as potential novel targets for the treatment of metastatic cancers.

The authors gratefully acknowledge support from NIH grant GM62431 (GGB) and the DOD Breast Cancer Training Grant (DV).

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Controlled Release Systems for Non-Viral Vectors

Lonnie D. Shea and Angela K. Pannier

Adapting controlled release technologies to the delivery of non-viral vectors has the potential to overcome barriers that limit gene therapy. Controlled release systems can enhance gene delivery and increase the extent and duration of transgene expression relative to more traditional delivery methods. Delivery vehicles for controlled release are fabricated from natural and synthetic polymers, which function either by releasing the vector into the local tissue environment or by maintaining the vector at the polymer surface. Vector release or binding is regulated by the effective affinity of the vector for the polymer, which depends upon the strength of molecular interactions. These interactions occur through non-specific binding based on vector and polymer composition or through the incorporation of complementary binding sites (e.g., biotin-avidin). This review examines the delivery of non-viral vectors from natural and synthetic polymers, and presents opportunities for continuing developments to increase their applicability.

Introduction

Controlled release systems for low molecular weight drugs and proteins have become a multi-billion dollar industry, with products such as...
Nutropin® Depot, Gliadel® wafer, Norplant, and CYPHER™ Stent. These systems illustrate the potential advantages of controlled release, which include: (1) maintained drug levels within a desirable range, (2) localized delivery to a target tissue or cell type to avoid adverse side effects, (3) decreased dose or number of dosages, and (4) facilitated delivery for fragile compounds (i.e., short half-lives). The adaptation of controlled release technologies to the delivery of non-viral vectors has the potential to overcome barriers that limit gene therapy. Controlled release can maintain elevated DNA concentrations in the cellular microenvironment, which improves gene delivery. Additionally, non-viral vectors may have a relatively short half-life, and delivery vehicles can either prevent their degradation or provide a sustained release. This review examines gene delivery from biomaterials and discusses how continuing advances will increase their applicability.

**Delivery mechanisms**

Controlled release systems typically employ polymeric biomaterials that deliver vectors according to two general mechanisms: i) polymeric release in which the DNA is released from the polymer or ii) substrate-mediated in which DNA is retained at the surface. For polymeric release, DNA is entrapped within the material and released into the environment, with release typically occurring through a combination of diffusion and polymer degradation. Polymeric delivery may enhance gene transfer by first protecting DNA from degradation, and then maintaining the vector at effective concentrations, extending the opportunity for internalization. DNA release into the tissue can occur rapidly, as in bolus delivery, or extend over days to months. Conversely, substrate-mediated delivery, also termed solid phase delivery, describes the immobilization of DNA to a biomaterial or extracellular matrix, which functions to support cell adhesion and places DNA directly in the cellular microenvironment. Cells cultured on the substrate can internalize the DNA either directly from the surface, or by degrading the linkage between the vector and the material.

**Vehicle formulations**

Vehicles for gene delivery can be fabricated from both natural and synthetic polymers and processed into a variety of forms, including nanospheres, microspheres, or scaffolds. Nanospheres are particles with diameters ranging from approximately 50 nm to 700 nm, consistent with the size of non-viral vectors. Nanoparticles are internalized and release DNA intracellularly. In contrast, microspheres with diameters ranging from 2 µm to 100 µm, are not readily internalized, but retained within the tissue to release DNA. Released DNA can transfect cells at the delivery site, with the protein product acting locally or distributed systemically. Alternatively, polymeric scaffolds function to define a three-dimensional space and can either be implanted or designed to solidify upon injection. These scaffolds can deliver DNA to cells within the surrounding tissue, or can target those infiltrating the scaffold.

A variety of natural and synthetic materials have been employed for DNA delivery, which can be categorized as either hydrophobic (e.g., poly(lactide-co-glycolide) (PLG), polyanhydrides) or hydrophilic polymers (e.g., hyaluronic acid (HA), collagen, poly(ethylene glycol) (PEG)). Synthetic polymers such as PLG and polyanhydrides have been widely used in drug delivery applications, as they are biocompatible and available in a range of copolymer ratios to control their degradation. Drug release from these polymers typically occurs through a combination of surface desorption, drug diffusion, and polymer degradation. Alternatively, hydrogels, which are often more than 98% water and maintain the activity of encapsulated vectors, released DNA by diffusion from the polymer network, which can be controlled by crosslinking the polymer.
**Naked DNA**

Naked DNA delivery by traditional mechanisms generally results in low but sustained expression *in vivo*, which is limited by poor uptake due to factors such as degradation and clearance. Physical (e.g., ultrasound, hydrodynamic injection) and chemical (e.g., cationic lipids/polymer) methods are continually being improved to enhance cellular uptake of naked DNA by altering cell permeability or enhancing cellular interactions. Nevertheless, polymeric delivery represents an alternative approach that can increase residence time within the tissue and protect against degradation.

Naked DNA interacts weakly with many polymers, leading to release from the vehicle with rates modulated by the polymer properties. Collagen-based materials released naked DNA *in vitro* for times ranging from hours to days, yet intramuscular implantation of collagen pellets maintained the DNA locally for 60 days. HA-based hydrogels also release the DNA; however, the rate of release can be controlled by the extent of crosslinking. For synthetic polymers such as PLG, the integrity of the DNA can be affected by degradation of the polymer to lactic acid and glycolic acid. PLG polymers can provide release rates ranging from a few days to more than 60 days (Figure 1), with the fabrication method and the polymer composition regulating release. Ethylene vinyl-co-acetate (EVAc) polymers can similarly provide a sustained release of DNA on the time scale of weeks.

DNA releasing polymers administered to multiple sites *in vivo* have demonstrated the capacity to transfected cells locally and promote sustained protein production. An injectable PLG formulation delivered subcutaneously led to 28 days of expression with 50 µg of DNA. An implantable PLG scaffold delivering 500 µg of DNA was able to transfect cells within and adjacent to the scaffold, and promote physiological responses. Collagen minipellets containing 50 µg of DNA administered intramuscularly elicited systemic effects for at least 60 days, which was significantly longer than direct DNA injection.

**DNA Complexes**

Although naked DNA provides transfection *in vivo*, packaging DNA with cationic lipids or polymers can enhance *in vivo* transfection. Complexes of naked DNA with cationic polymers or lipids facilitate cellular internalization, by creating a less negative surface charge and providing stability against degradation. The presence of complexation agents can also maintain the stability of DNA complexes during polymer processing, and in some cases increase encapsulation efficiency. Porous PLG or collagen scaffolds with encapsulated polyplexes or lipoplexes achieved substantial transfection *in vitro* and *in vivo*, but with significantly altered release profiles compared to naked DNA, due to interactions of the complexation reagents with the biomaterial or with adsorbed serum components.

Interactions between complexation agents and the polymer have been adapted to specifically immobilize DNA complexes to a substrate. Poly(L-lysine) (PLL) and PEI were modified with biotin residues for subsequent complexation with DNA and binding to a neutravidin substrate. Poly(L-lysine) (PLL) and PEI were modified with biotin residues for subsequent complexation with DNA and binding to a neutravidin substrate.
mixtures of biotinylated and non-biotinylated cationic polymer at a constant N/P ratio. For complexes formed with PLL, the number of biotin groups and their distribution among the cationic polymer were critical determinants of both binding and transfection (Figure 2). Increasing the number of biotin groups per complex led to increased binding. However, transfection was maximal when complexes contained biotin residues attached to a small fraction of the cationic polymers. At this condition, less than 100 ng of immobilized DNA mediated transfection, which was increased 100 fold relative to bolus delivery of similar complexes. For complexes formed with PEI, substantial transfection was observed, but was independent of the number of biotin groups present on the complex, which suggests that complex binding occurred by non-specific interactions with the substrate. Other systems have used non-specific binding to mediate delivery. PLGA and collagen membranes were coated with phosphatidyl glycerol (1-5%) to support binding of complexes formed with polyamidoamine (PAMAM) dendrimers. In vivo studies demonstrated a six to eight-fold enhancement in transfection relative to naked DNA delivery.

**Applications**

**Gene Therapy:** Numerous clinical trials have been completed or are pending for a multitude of pathologies including malignancy (e.g., colorectal, bladder, and brain). Most trials have not shown significant therapeutic efficacy or clinically useful responses, likely due in part to inefficient gene transfer. Polymeric-based gene delivery systems may enhance delivery of the vector and extend the duration of transgene expression to achieve sufficient protein quantities that act locally or systemically. For example, IL-2, IL-12, and TNF-α expression induced by a DNA releasing gelatin sponge inhibited tumor growth in heterotopic nodules of tumor bearing mice.

**Functional Genomics:** Transfected cell arrays represent a high throughput approach to correlate gene expression with functional cell responses, based on gene delivery from a surface. In principle, this system can be employed for numerous studies, such as screening large collections of cDNAs or targets for therapeutic intervention. Transfected cell arrays were formed using a substrate-mediated approach in which plasmids or adenoviruses were mixed with collagen and spotted onto glass slides or into wells. Piated cells were transfected and could be analyzed for cellular responses using a variety of imaging or biochemical techniques.
Conclusions

In comparison to traditional gene delivery systems, controlled release can enhance gene delivery by increasing the extent and duration of transgene expression, while reducing the need for multiple interventions. These polymer-based gene delivery systems capitalize on both specific and non-specific interactions between the biomaterial and vector, to achieve either release into the extracellular space or immobilization at the surface. While the potential to use these polymeric systems has been established, the design parameters by which to optimize or control gene transfer are not well understood. Vector and biomaterial development, combined with studies that correlate system properties (e.g., dose, release rate) with the extent of transgene expression (i.e., quantity and duration of protein produced, location of transgene expression) will lead to molecular scale design of delivery systems. The development of these systems may increase the efficacy within current gene therapy trials, and may also extend the applicability of gene delivery to other areas such as functional genomics.

Acknowledgments

We would like to thank Tatiana Segura, Zain Bengali, and Tiffany Houchin for their critical evaluation of the manuscript. Support was provided by the Specialized Program of Research Excellence (SPORE) in Breast Cancer (P50-CA89018) and NSF (BES0092701 (LDS), Graduate Fellowship (AKP)).

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17. R. E. Elaz, E. C. Nisk, Jr., Gene Ther 9, 1230-7 (Sep, 2002).
Improvements in oncology treatment are increasingly focused on reducing patient burden in terms of side effects, convenience, and personal cost, in addition to traditional concerns of tumor response and survival. Evaluation of the importance of such advances requires measurement of patient preference and tolerance for side effects of treatment. Partial breast radiotherapy may be a treatment modality that offers tumor control similar to traditional breast radiotherapy while reducing the burden of therapy. In our study, we evaluated patient-reported quality of life for women early in their radiation treatment for breast cancer. Our study demonstrates the varied sensitivity to change of multiple quality of life measures, and identifies the early quality of life impact of radiation treatment.
Background

In early stage breast cancer, the use of lumpectomy combined with radiotherapy is a standard form of therapy. Traditional breast radiotherapy includes 5-7 weeks of daily radiation. The target of the radiotherapy traditionally includes the entire breast. It has been known for some time that the majority of recurrences after lumpectomy occur in the region of the lumpectomy cavity. There is increasing data that radiotherapy delivered only to the lumpectomy cavity may offer similar control rates as traditional radiotherapy. The more limited radiotherapy can be delivered in only 10 treatments over one week. Therefore, partial breast radiotherapy may offer reduced toxicity related to the smaller radiotherapy field and possibly improved quality of life (QoL).

Patients report a wide range of toxicities related to radiation treatment. Potential physical side effects, both acute and persisting, commonly include irritation of the skin of the breast, breast pain, lymphedema, radiation dermatitis, pneumonitis, cardiac damage and fatigue. Patients reported varied emotional responses during radiation therapy, including tension, loneliness, nervousness, anxiety, and/or depression. A wide range of instruments are used to measure quality of life. Some measures focus on specific factors such as pain (Modified Post-operative Pain Questionnaire, Pain Disability Index, McGill Pain Questionnaire) and mental health (Mental Health Inventory), while others capture multi-dimensional aspects of patient well-being (Functional Assessment of Cancer Therapy and the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire). Comparative studies have further demonstrated the adverse impact of radiation therapy on quality of life. Quality of life declines over the course of radiation therapy (measured by the Quality of Life Index, a 5-item questionnaire, Dow & Lafferty; and a modified version of the Breast Cancer Chemotherapy Questionnaire, Whelan et al.) even as it improves in women with lumpectomy alone.

Each of the published studies has relied on quality of life measures that query patients about the presence and severity of specific side effects or aspects of patient well-being. Still broader measures of quality of life allow patients to consider all aspects of their health and well-being, and to weight these factors in accordance with their own preferences. One approach to measuring quality of life is to assess a subject’s utility, or “satisfaction” for their current health. Utilities are preference-based measures of quality of life that range from 0 (current health is equivalent in value to death) to 1 (current health is equivalent in value to perfect health). These measures are commonly used to weight survival in cost-effectiveness studies. For example, 10 years spent in perfect health is equal to 10 quality-adjusted life years, but only 5 quality-adjusted life years if the utility of that time was equal to 0.5.

Many methods exist and are commonly used for assessing utilities, including a standard gamble instrument, and a time trade-off instrument. The standard gamble has a sound basis in expected utility theory, the accepted normative theory of choice. The standard gamble (SG) includes a feature of risk-taking, which is inherent in all medical decision-making. Participants consider a choice between staying in their current health for the rest of their lives (or staying in some described hypothetical health state), or undergoing an imaginary treatment with no side effects that would restore them to perfect health, but carries with it a stated risk of immediate death. The time trade-off (TTO) method of assessing utility scores was developed by Torrance and his colleagues specifically for utility assessment in health care. Rather than asking respondents to consider choices with immediate outcomes, participants are asked whether they would be willing to trade time to attain perfect health. For example, in a typical
TTO interview, participants may be asked what proportion of a year spent in perfect health is equivalent to a year spent in the described hypothetical health state. The length of time may be constant across all respondents, or may be life expectancy-adjusted. Many alternatives exist to the standard gamble and time trade-off instruments. A visual analog scale is often used, which simply asks respondents to rate their overall quality of health on a linear scale. Also, significant effort has gone into preference-rated (or weighted) instruments for assessing current health utility scores, such as the Quality of Well-Being Index17, Euroqol18 and Health Utilities Index19. Each asks a series of health status questions, and then converts the health status score to a utility score, based on scoring systems derived from community evaluations of hypothetical health states.

Taking quality of life into account, past studies have evaluated the relative cost-effectiveness of radiation therapy when compared with other medical interventions. Using a Markov model, Hayman et al.12, compared the strategy conservative surgery (CS) with or without radiation therapy (RT). Using the standard gamble utility measure, both breast cancer patients treated with lumpectomy followed by RT, and medical oncology nurses rated five different health states related to breast cancer treatment, on a scale that ranged from 0 (equivalent to death) to 1 (perfect health). Utility scores ranged from 0.81 (CS alone with an isolated local recurrence salvaged with mastectomy and reconstructive surgery) to 0.92 (CS and RT without local or distant recurrence). They found that the addition of RT resulted in a $9,800 per patient cost increase, and an increase of 0.35 quality adjusted life years (QALYs), an incremental cost effectiveness ratio of $28,000/QALY, which was cost effective compared with other accepted medical interventions. In an earlier study, using the same utility scores elicited of breast cancer patients and medical oncology nurses cited above, Hayman et al. found that both fear of local future recurrence and actual recurrence leading to mastectomy have such a detrimental impact on QoL that patients are willing to accept the risks and inconvenience of RT to avoid them20. The investigators concluded that early-stage breast cancer patients who valued breast preservation rated the benefits of RT after breast-conserving surgery to outweigh both the risks of RT and its potential negative impact on quality of life. While one of these studies modeled the cost effectiveness of radiation therapy and the other demonstrated patient preference for treatment, a full understanding of the overall QoL impact of radiation therapy is required using a preference-based measure for the calculation of its cost-effectiveness relative to alternative therapies.

Methods
Breast cancer patients undergoing radiation treatment at Northwestern Memorial Hospital were recruited for participation in the study. All patients receiving radiation treatment for breast cancer were considered eligible. After obtaining informed consent, a baseline interview was conducted with each patient following either her 3rd, 4th or 5th radiation treatment (week 1), with a follow up interview after either her 8th, 9th or 10th radiation treatment (week 2). During both interviews, participants completed the patient-rated Eastern Cooperative Oncology Group (ECOG) performance status rating. Participants were then randomized to one of two groups, which determined the order in which quality of life surveys were given, which included the Functional Assessment of Cancer Therapy – Breast (FACT-B). The FACT-B is a well-established multidimensional QOL instrument21,22. Patients also completed standard gamble and time trade-off utility questionnaires at both assessments.

Multiple outcome measures were used to describe different aspects of patient quality of life. The TTO and SG questionnaires provide preference-based measures of patient utility.
The FACT-B was summarized in to 5 measures: physical, functional, emotional and social/family well-being, plus the breast cancer subscale, which assesses specific symptoms and concerns of women diagnosed and treated for breast cancer. The ECOG performance score was used as a primary measure of patient function.

Descriptive analyses were conducted to characterize quality of life during the first 2 weeks of radiation treatment. Changes in quality of life were modeled using a linear regression model, to determine whether patient characteristics (baseline function, age and living arrangements) could predict improvement or worsening of quality of life, early in radiation treatment. All statistical analyses were conducted at the $\alpha = 0.10$ level.

**Results**

A total of sixty-nine patients completed both the baseline and follow-up interviews. The patients ranged in age from 26 to 83 years (mean = 54 years), and were primarily insured privately (fee for service or preferred provider organization). Quality of life at baseline varied considerably across all study measures. Table 1 summarizes baseline measures of function and quality of life.

<table>
<thead>
<tr>
<th></th>
<th>Mean (Medium)</th>
<th>Std Dev</th>
<th>Min/Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECOG Perf Status</td>
<td>0.362 (0)</td>
<td>0.613</td>
<td>0/2</td>
</tr>
<tr>
<td>Standard Gamble</td>
<td>0.937 (0.988)</td>
<td>0.098</td>
<td>0.45/1.00</td>
</tr>
<tr>
<td>Time Trade-Off</td>
<td>0.934 (0.988)</td>
<td>0.082</td>
<td>0.65/1.00</td>
</tr>
<tr>
<td>Functional WB</td>
<td>22.362 (24)</td>
<td>5.228</td>
<td>1/28</td>
</tr>
<tr>
<td>Emotional WB</td>
<td>19.739 (20)</td>
<td>3.599</td>
<td>10/24</td>
</tr>
<tr>
<td>Social/Family WB</td>
<td>23.829 (25)</td>
<td>5.663</td>
<td>0/28</td>
</tr>
<tr>
<td>Breast Cancer Concerns</td>
<td>25.265 (25.875)</td>
<td>6.648</td>
<td>1.125/36</td>
</tr>
</tbody>
</table>

Table 1: Week 1 QOL Measures

Patient function declined between week 1 and 2, as measured by the patient-reported ECOG performance status (Figure 1; $p < 0.001$).

No change in QOL was detected by the four domains of the FACT-B (physical, functional, emotional, and social/family well-being; $p > 0.10$). However, improvement was documented by the breast cancer subscale (from 25.31 to 26.39, $p < 0.01$) and both utility measures ($p < 0.01$; Figure 2).

Multiple regression models were conducted to determine predictors of improvement in QOL between weeks 1 and 2. Statistically significant predictors of change differed across the 3 models. Improvement in standard gamble scores was weakly related to higher baseline ECOG performance status (no symptoms relative to some bed rest) while improvement in breast cancer subscale scores was more substantially related to lower baseline ECOG performance status (some symptoms relative to none). Improvement in time trade-off scores was related to the presence of other adults in the home (a measure of social support).
Discussion

Our QOL instruments measure QOL in very different ways. The FACT-B poses specific questions to the patients about aspects of their well-being. Four of the subscales are designed to generalize to all cancer patients, and the fifth addresses specific concerns for women with breast cancer. The utility questionnaires ask patients to think broadly about their overall quality of life, without delineation of specific factors of their well-being that might be affected by cancer or its treatment.

The present study was originally planned as a small pilot to test the use of multiple quality of life instruments in a sample of women early in radiation treatment. Because no medical chart review data was collected, it is not possible to characterize the clinical status of the patients in our study. Clinical indicators including stage of disease, prior treatment, and comorbidities would be expected to increase the explanatory power of our models. Although the study patients represent a diverse group with respect to age, living arrangements and insurance payor, they each received care at a single institution. Our study findings may not be generalizable to the population of breast cancer patients receiving radiation treatment.

Our study demonstrated considerable variation in the quality of life of women early in radiation treatment for breast cancer. Using multiple QOL instruments, we found seemingly contradictory results – worsening in ECOG performance status with either no change or improvement in quality of life – between the first two weeks of treatment. Both measures of utility, the standard gamble and time trade-off, documented small (less than one third of a standard deviation) improvements in overall quality of life. Although these improvements were statistically significant, they are unlikely to be judged clinically important. The improvements in quality of life were generally not well-predicted by baseline characteristics of the patients. Breast cancer subscale scores also improved, though again by a small amount.

However, the finding that patient-reported quality of life did not worsen early in radiation treatment, even as ECOG performance status did, suggests that there may be little or no adverse affect of radiation treatment on patients’ quality of life. Statistically significant improvements in symptoms identified by the breast cancer subscale included swollen arms, bother from hair loss, and bother from change in weight. It is likely (though not tested in this study) that improvements in quality of life indicate an adjustment in expectations of treatment effects by the patients, rather than a true reduction in symptoms.

Currently there is significant interest in accelerated partial breast radiotherapy (PBRT). PBRT can be delivered with brachytherapy or external beam radiotherapy. Brachytherapy involves an invasive procedure to place needles or a balloon catheter into the area of the lumpectomy.

<table>
<thead>
<tr>
<th></th>
<th>Change in Standard Gamble Score</th>
<th>Change in Time Trade-Off Score</th>
<th>Change in Breast Cancer Subscale Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td>0.001</td>
<td>0.000</td>
<td>0.041</td>
</tr>
<tr>
<td>Living with other adults vs. Alone</td>
<td>0.017</td>
<td>0.035</td>
<td>0.064</td>
</tr>
<tr>
<td>ECOG: Some symptoms vs. Normal</td>
<td>-0.007</td>
<td>0.019</td>
<td>0.267</td>
</tr>
<tr>
<td>ECOG: Some bedrest vs. Normal</td>
<td>-0.072</td>
<td>0.010</td>
<td>0.858</td>
</tr>
<tr>
<td>R²</td>
<td>0.133</td>
<td>0.064</td>
<td>0.121</td>
</tr>
</tbody>
</table>

*Constant not reported

Table 2. Prediction of Change in QOL
Radiotherapy in PBRT is generally delivered two times per day for one week. It has been thought that treatment delivered rapidly over one week would be convenient and therefore have the potential to improve overall QoL. This current study found a small improvement in QoL during the second week of standard breast radiotherapy. In addition, when brachytherapy is utilized for PBRT there may be a further decrement in QoL given the invasiveness of the procedure. As we move forward in the use of PBRT it is important to prospectively measure QoL in addition to standard toxicity and efficacy.

**REFERENCES**


The Robert H. Lurie Comprehensive Cancer Center of Northwestern University funds 18 shared facilities and resources that provide services, equipment and expertise that are fundamental to understanding the basic biology and clinical manifestations of cancer. These facilities and resources are accessible to all of the members of the Cancer Center and support the Cancer Center’s mission to foster basic and translational research in the mechanisms and treatment of cancer. To find out more information about the Shared Research Core Facilities, visit the Cancer Center’s Web site at http://www.cancer.northwestern.edu/research.cfm. Two of the 18 Shared Research Core Facilities are highlighted below.

Pathology Core Facility
The Pathology Core Facility of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University has three main components: research histology, specimen procurement and oversight of clinical trials.

The research histology component provides all of the tissue processing and histology services typically performed in a clinical laboratory but it is specifically dedicated to the needs of the Northwestern University research community in general and the Cancer Center research community in particular. The Pathology Core Facility is unique in that it has the capability and flexibility to address specific research protocol needs.

The tissue procurement component of the Pathology Core Facility has two main functions – human tissue and fluid procurement, storage and distribution and quality assurance and protection of research subjects. The facility is directed by Michael R. Pins, MD. You can reach him via phone (312) 908-9595 or via email m-pins@northwestern.edu.

Flow Cytometry Core Facility
The Flow Cytometry Core Facility of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University provides comprehensive flow cytometry and cell sorting services for investigators of the Cancer Center, Northwestern University’s Feinberg School of Medicine, Northwestern University and other affiliated institutions. In addition to providing access to routine flow cytometry assays such as immunophenotyping and DNA analysis, the facility provides the guidance, technical
assistance, and equipment for investigators to utilize more complex multi-parametric, multi-laser measurements as well as cell sorting in their research. Thus, the Flow Cytometry Core Facility serves as a focus for individuals interested in cellular based measurements and cellular heterogeneity in disease.

Services provided by the facility personnel extend from consultation on experimental design, sample preparation and data analysis to instrument operation and set-up for cell-sorting and multi-laser operation. The facility is directed by Charles Goolsby, PhD. You can reach him via phone (312) 908-1294 or via email c-goolsby@northwestern.edu. In addition, you may contact Jeff Nelson, technologist at j-nelson3@northwestern.edu or Mary Paniagua, manager at m-paniagua@northwestern.edu.

The inhibins are gonadal transforming growth factor β superfamily protein hormones that suppress pituitary follicle-stimulating hormone (FSH) synthesis. Recently, betaglycan and inhibin binding protein (InhB/P/p120, also known as the product of immunoglobulin superfamily gene 1 [IGSF1]) were identified as candidate inhibin coreceptors, shedding light on the molecular basis of how inhibins may affect target cells. Activins, which are structurally related to the inhibins, act within the pituitary to stimulate FSH production. Betaglycan increases the affinity of inhibins for the activin type II receptor (ACVR2) receptor, thereby blocking activin binding and signaling through this receptor. InhB/P/p120 may not directly bind inhibins but may interact with the activin type IB receptor, ALK4, and participate in inhibin B’s antagonism of activin signaling. To better understand the in vivo functions of InhB/P/p120, we characterized the InhB/P/p120 mRNAs and gene in mice and generated InhB/P/p120 mutant mice by gene targeting in embryonic stem cells. InhB/P/p120 mutant male and female mice were viable and fertile. Moreover, they showed no alterations in FSH synthesis or secretion or in ovarian or testicular function. These data contribute to a growing body of evidence indicating that InhB/P/p120 does not play an essential role in inhibin biology.

IGF-1 and MAP Kinase Involvement in the Stimulatory Effects of LNCaP Prostate Cancer Cell Conditioned Media on Cell Proliferation and Protein Synthesis in MC3T3-E1 Osteoblastic Cells. Journal of Cellular Biochemistry, Vol. 90: pp.925-937, October 2003 (this material is used by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.).

Bone metastases form prostate cancer cause abnormal new bone formation, however, the factors involved and the pathways leading to the response are incompletely defined. We investigated the mechanisms of osteoblast stimulatory...
effects of LNCaP prostate carcinoma cell conditioned media (CM). MC3T3-E1 osteoblastic cells were cultured with CM from confluent LNCaP cells. LNCaP CM stimulated MAP kinase, cell proliferation (³H-thymidine incorporation), and protein synthesis (¹⁴C-proline incorporation) in the MC3T3-E1 cells. The increases in cell proliferation and protein synthesis were prevented by inhibition of the MAP kinase pathway. IGF-I mimicked the effects of the CM on the MC3T3-E1 cells and inhibition of IGF-I action decreased the LNCaP CM stimulation of ³H-thymidine and ¹⁴C-proline incorporation and MAP kinase activity. The findings indicate that IGF-I is an important factor for the stimulatory effects of LNCaP cell CM on cell proliferation and protein synthesis in osteoblastic cells, and that MAP kinase is a component of the signaling pathway for these effects.


Transformation of human cells requires both SV40 large T and small t antigens. Plasmids that contained mutations in the amino-terminal dnaJ domain of the early region fail to transform human diploid fibroblasts. However, large T dnaJ mutants can be rescued by plasmids that express early region products other than large T antigen. The protein found to be responsible for such complementation was the third early region product, 17KT. Similar to large T, this protein reduces levels of the retinoblastoma-related protein, p130, and stimulates cell-cycle progression of quiescent fibroblasts, two activities of large T that are disrupted by dnaJ mutations.

Chatterton RT, Geiger AS, Gann PH and Khan SA

The study was designed to determine the process and limitations by which estrone sulfate may be a precursor of estradiol in the parenchymal cells of the normal breast. The concentration of estrone sulfate in breast nipple aspirate fluid was 1000-fold greater than that of estradiol. Concentrations of ³H-estrone sulfate in parenchymal cells were only 0.20-0.33 times that of the 1.0nM concentration in the medium, while ³H-estrone achieved concentrations up to 24 times that in the medium at 37⁰C. Nevertheless, estrone sulfate added to the medium was linearly converted within a 1000-fold concentration range to estrone in intact cells with a mean half-time of conversion of 628 min per 10⁶ cells. Homogenized cells had a half-time of 246 min per 10⁶ cells. This, the time for entry of estrone sulfate into cells reduced the rate by approximately 55%. In split samples, the vₐₘₚ values (±S.D.) for intact and homogenized cells were 12.6 ± 1.4 and 18.3 nmol/h mg DNA, respectively (P<0.03). The corresponding kₘ values for intact and homogenized cells were 6.0 ± 1.1 and 4.7 ± 1.0 µM.

Conversion of estrone sulfate to estradiol was more efficient in intact cells than in homogenates with mean half-times of 2173 and 7485 min per 10⁶ cells, respectively. Conversion of estrone to estrone sulfate did not occur in these cells despite sulfonation of estrone by MCF-7 breast cancer cells under identical conditions. It is concluded that estrone sulfate can serve as a
precursor for estradiol in normal breast tissue. Conversion of estrone to estradiol is a limiting step in the process.

Frater JL, Yaseen NR, Peterson LC, Tallman MS and Goolsby CL


Acute leukemias demonstrating immunophenotypic features of more than 1 cell lineage are referred to as acute leukemias of ambiguous lineage in the new World Health Organization classification system. A subtype of leukemia of ambiguous lineage is biphenotypic acute leukemia in which the malignant cell population expresses markers of 2 different lineages, most commonly myeloid and either B- or T-lymphoid lineages. This entity has been defined by a scoring system proposed by the European Group for the Immunological Characterization of Acute Leukemias (EGIL), with various markers assigned a score of 2, 1, or 0.5 depending on their specificity for myeloid or lymphoid lineage. Those cases having a score greater than 2 for the myeloid and either the B- or T-lymphoid lineages are biphenotypic acute leukemia in this system. One marker, CD79a, has been so clearly associated with acute lymphoblastic leukemia (ALL) by some researchers that its expression in the presence of blast markers is considered indicative of B-ALL. We describe an unusual case of acute leukemia meeting the criteria for biphenotypic acute leukemia in which CD79a expression was observed in the blast population.


Purpose: Cancer patients who are deficient in literacy skills are particularly vulnerable to experiencing different outcomes due to disparities in care or barriers to care. Outcomes measurement in low literacy patients may provide new insight into problems previously undetected due to the challenges of completing paper and pencil forms.

Description of study: A multimedia program was developed to provide a quality of life assessment platform that would be acceptable to patients with varying literacy skills and computer experience. One item at a time is presented on the computer touchscreen, accompanied by a recorded reading of the question. Various colors, fonts and graphic images are used to enhance visibility, and a small picture icon appears near each text element allowing patients to replay the sound as many times as they wish. Evaluation questions are presented to assess patient burden and preferences.

Results: A ethnically diverse group of 126 cancer patients with a range of literacy skills and computer experience reported that the “talking touchscreen” (TT) was easy to use, and commented on the usefulness of the multimedia approach.

Clinical implications: The TT is a practical, user-friendly data acquisition method that provides greater opportunities to measure self-reported outcomes in patients with a range of literacy skills.
Haynes SA, Huang X, Kambhampati S, Plataniotis LC and Bergan RC


Transforming growth factor beta (TGFβ) regulates cell adhesion, proliferation, and differentiation in a variety of cells. Smad proteins are receptor-activated transcription factors that translocate to the nucleus in response to TGFβ.

We demonstrate here that TGFβ increases cell adhesion in metastatic PC3-M prostate cancer cells. TGFβ treatment of PC3-M cells leads to nuclear translocation of R-Smad proteins. We show that Smad proteins are necessary, but not sufficient, for TGFβ-mediated cell adhesion.

After showing that TGFβ upregulated p38 MAP kinase activity in PC3-M cells, we show that inhibition of p38 MAP kinase partially blocked TGFβ-mediated increase in cell adhesion, as well as nuclear translocation of Smad3. Finally, we show that Smad3 is phosphorylated by p38 MAP kinase in vitro. These findings implicate crosstalk between the MAP kinase and Smad signaling pathways in TGFβ’s regulation of cell adhesion in human prostate cells. This represents a mechanism by which the pleiotropic effects of TGFβ may be channeled to modulate cell adhesion.

Jeruss JS, Sturgis CD, Rademaker AW and Woodruff TK


Activin and transforming growth factor (TGF)-β members of the TGF-β superfamily of growth factors, have been implicated in both mammary gland development and breast carcinogenesis. TFG-β is thought to be involved in the maintenance of mammary gland ductal architecture and postlactational involution. TGF-β acts as both a tumor suppressor and has oncogenic capacities in breast cancer tissue. Activin is associated with the growth modulation in glandular organs, and its receptors and signaling proteins are present and regulated during postnatal mammary gland development, primarily during the lactational phase. The presence of the major components of the activin signal transduction pathway in different pathologic grades of breast cancer tissue has not been described thoroughly, despite evidence from in vitro studies suggesting that activin can inhibit proliferation in breast cancer-derived cells. On the basis of the growth regulatory capacity of activin, we hypothesized that the components of this signal transduction system would be deregulated as breast cancer becomes more aggressive. To test this hypothesis, breast cancer samples were substratified by pathologic grade, a known prognostic marker for breast cancer, and then examined for the presence and cellular localization of activin ligand subunits (βA- and βB), receptors (Act RIα, Act RIβ, and Act RIβ), and signaling proteins, Smads 2, 3, and 4, by immunohistochemistry and immunofluorescent analysis. Breast tissue from healthy patients undergoing reduction mammoplasty was also studied. The activin βA-subunit was present in all of the tissues examined, whereas the βB-subunit, activin type II receptors, and Smads were less evident in high-grade cancers. Significant correlations were made in breast cancer specimens between a decrease in nuclear Smad 3 abundance and high tumor grade, high architectural grade, larger tumor size and hormone receptor negativity. Thus, activin signal transduction components are present in normal tissue and grade 1 cancer but down-regulated in high-grade cancer. The deregulation of this signal transduction system may be relevant to advancing oncogenic progression.
Perry K and Mondragón A

**Structure of a Complex Between E. coli DNA Topoisomerase I and Single-Stranded DNA.**


In order to gain insights into the mechanism of ssDNA binding and recognition by *Escherichia coli* DNA topoisomerase I, the structure of 67 kDa N-terminal fragment of topoisomerase I was solved in complex with ssDNA. The structure reveals a new conformational stage in the multistep catalytic cycle of type IA topoisomerases. In the structure, the ssDNA binding groove leading to the active site is occupied, but the active site is not fully formed. Large conformational changes are not seen; instead, a single helix parallel to the ssDNA binding groove shifts to clamp the ssDNA. The structure helps clarify the temporal sequence of conformational events, starting from an initial empty enzyme and proceeding to a ssDNA-occupied and catalytically competent active site.

Portis T, Dyck P and Longnecker R

**Epstein-Barr Virus (EBV) LMP2A Induces Alterations in Gene Transcription Similar to Those Observed in Reed-Sternberg Cells of Hodgkin Lymphoma.**


Epstein-Barr virus (EBV) is associated with the development of a variety of malignancies, including Hodgkin lymphoma. One of the few viral transcripts expressed in EBV-positive Hodgkin/Reed-Sternberg (HRS) cells of Hodgkin lymphoma is latent membrane protein 2A (LMP2A). This viral protein blocks B-cell receptor (BCR)-signaling in vitro. Furthermore, expression of LMP2A in developing B-cells in vivo induces a global down-regulation of genes necessary for proper B-cell development. In this study we have analyzed gene transcription in primary B cells from LMP2A transgenic mice, LMP2A-expressing human B-cell lines, and LMP2A-positive and –negative EBV-infected lymphoblastoid cell lines (LCLs). We demonstrate that LMP2A increases the expression of genes associated with cell cycle induction and inhibition of apoptosis, alters the expression of genes involved in DNA and RNA metabolism, and decreases the expression of B-cell-specific factors and genes associated with immunity. Furthermore, many alterations in gene expression induced by LMP2A are similar to those recently described in HRS cells of Hodgkin lymphoma and activated, proliferating germinal center centroblasts/centrocytes. These correlations suggest that LMP2A expression in EBV-infected B cells may lead to the induction and maintenance of an activated, proliferative state that could ultimately result in the development of Hodgkin lymphoma.

Staradub VL, Rademaker AW and Morrow M

**Factors Influencing Outcomes for Breast Conservation Therapy of Mammographically Detected Malignancies.**


Objective: To evaluate the importance of surgeon caseload, lesion type and biopsy type on outcomes in breast conservation therapy (BCT).

Background: Breast conservation therapy has low rates of morbidity and mortality and is being performed with increasing frequency. Its primary advantage is cosmetic, and the amount of breast tissue resected is the main determinant of cosmetic outcomes.

Study Design: Two hundred seventeen consecutive patients undergoing breast conservation therapy at Northwestern Memorial Hospital for mammographically detected breast cancer were evaluated. The volume of tissue excised was...
compared with the volume of the tumor as a ratio. Univariate and multivariate analyses of the relationships between the specimen-to-tumor-volume ratio (STVR) and histologic diagnosis, biopsy type, surgeon caseload and lesion type were examined.

Results: The mean (log scale) STVR was significantly lower when the mammographic lesion was identified as a mass or architectural distortion versus calcifications (p<0.001 in multivariate analysis). Mean log (STVR) was also decreased for higher-caseload surgeons (p=0.02). Core biopsy before lumpectomy was associated with significantly increased mean log (STVR) (83 versus 50, p=0.05) without significantly increasing the rate of negative margins.

Conclusions: Mammographic lesion type and biopsy method were associated with the amount of tissue excised relative to tumor size as measured by STVR. In addition, surgeons with higher caseloads were better able to perform needle localization lumpectomy to negative margins while limiting the volume of normal breast tissue excised.

Swanson KA, Kang RS, Stamenova SD, Hicke L and Radhakrishnan I


Monoubiquitylation is a well-characterized signal for the internalization and sorting of integral membrane proteins to distinct cellular organelles. Recognition and transmission of monoubiquitin signals is mediated by a variety of ubiquitin-binding motifs such as UIM, UBA, UEV, VHS and CUE in endocytic proteins. The yeast Vps27 protein requires two UIMs for efficient interactions with ubiquitin and for sorting cargo into multivesicular bodies. Here we show that the individual UIMs of Vps27 exist autonomously folded α-helices that bind ubiquitin independently, noncooperatively and with modest affinity. The Vps-27 N-terminal UIM engages the Leu8-Ile44-Val70 hydrophobic patch of ubiquitin through a helical surface conserved in UIMs of diverse proteins, including that of the S5a proteasomal regulatory subunit. The Leu8-Ile44-Val70 ubiquitin surface is also the site of interaction for CUE and UBA domains in endocytic proteins, consistent with the view that ubiquitin-binding endocytic proteins act serially on the same monoubiquitylated cargo during transport from cell surface to the lysosome.

Tsuruta D, Hopkinson SB, Lane KD, Werner MC, Cryns VL and Jones JCR


Within each hemidesmosome, α6β4 integrin plays a crucial role in hemidesmosome assembly by binding to laminin-5 in the basement membrane zone of epithelial tissue. Recent analyses have implicated “specificity-determining loops” (SDLs) in the I-like domain of β integrin in regulating ligand binding. Here, we investigated the function of an SDL-like motif within the extracellular I-like domain of β4 integrin. We generated point mutations within the SDL of β4 integrin tagged with green fluorescent protein (GFP-β4K150A and GFP-β4155L). We also generated a mutation within the I-like domain of the β4 integrin, lying outside the SDL region (GFP-β4V284E). We transfected constructs encoding the mutated β4 integrins and a GFP-conjugated wild type β4 integrin (GFP-β4WT) into 604G cells, which
assemble hemidesmosomes, and human endothelial cells, which express little endogenous β4 integrin. In transfected 804G cells, GFP-β4WT and GFP-β4V284E colocalize with hemidesmosome proteins, whereas hemidesmosomal components in cells expressing GFP-β4K150A and GFP-β4Q155L are aberrantly localized. In endothelial cells, GFP-β4WT and mutant proteins are co-expressed at the cell surface with α6 integrin. When transfected endothelial cells are plated onto laminin-5 matrix, GFP-β4K150A and GFP-β4Q155L do not. GFP-β4WT and GFP-β4V284E expressed in endothelial cells associate with the adaptor protein Shc when the cells are stimulated with laminin-5. However, GFP-β4K150A and GFP-β4Q155L fail to associate with Shc even when laminin-5 is present, thus impacting downstream signaling. These results provide evidence that the SDL segment of the β4 integrin subunit is required for ligand binding and is involved in outside-in signaling.

Winchester DJ, Bernstein JR, Jeske JM, Nicholson MH, Hahn EA, Goldschmidt RA, Watkin WG, Sener SF, Bilimoria MB, Barrera E and Winchester DP


Background: Nonpalpable mammographic abnormalities are frequently evaluated by means of a stereotactic core needle biopsy. This technique is a very sensitive indicator of invasive cancer, but is less reliable to discriminate between ductal carcinoma in situ and atypical ductal hyperplasia (ADH). The objective of this study was to determine the correlation of the 11-gauge vacuum-assisted core needle biopsy to open biopsy when a diagnosis of ADH is obtained.

Hypothesis: The use of 11-gauge vacuum-assisted stereotactic core needle biopsy does not conclusively diagnose ADH.

Design: Retrospective analysis.

Setting: University-affiliated teaching hospital.

Patients: Mammographic findings were evaluated with an 11-gauge vacuum-assisted stereotactic core biopsy in 1750 patients. Seventy-seven patients were diagnosed as having ADH; of these, 65 underwent excisional biopsy.

Main Outcome Measures: Pathological upstaging rate.

Results: Of the 65 patients who underwent excisional breast biopsy, 11 (17%) had their condition upstaged to a breast cancer diagnosis. These patients had presented at a later age than those who retained a benign diagnosis after excisional biopsy. The number of core taken did not correlated with diagnostic accuracy.

Conclusions: Of the 65 patients who underwent open biopsy for ADH in this series, only 83% had an accurate diagnosis. A diagnosis of ADH by stereotactic core needle biopsy should be followed by an open excisional biopsy.

Xiao W, Zhang Q, Jiang F, Pins M, Kozlowski JM and Wang Z


Androgens control prostate homeostasis and regulated androgen response genes. Here, we report the identification and characterization of U19, a novel testosterone-regulated apoptosis inducer with tumor suppressive activity. U19 is an evolutionarily conserved protein expressed in many human tissues, with the most abundant expression in the prostate, bone marrow, kidney
and lymph nodes. Overexpression of U19 in 12 surveyed cell lines induced apoptosis, and a new protein synthesis is required for apoptosis induction. Expression of U19 in xenograft prostate tumors markedly induced apoptosis and inhibited tumor growth in vivo. Consistent with its tumor-suppressive role, U19 down-regulation was observed in all of the surveyed prostate cancer cell lines and in 19 of 23 clinical human prostate tumor specimens. Loss of heterozygosity analysis revealed U19 allelic loss in 19 of the 23 specimens. Furthermore, two of the specimens had homozygous U19 deletions, and one specimen had hypermethylated U19 promoters, indicating that U19 can be inactivated genetically or epigenetically. These observations suggest that U19 is growth inhibitory and tumor suppressive and that the disruption of androgen-dependent growth inhibition via U19 down-regulation is commonly associated with prostate cancer progression.


Tumor angiogenesis, a major requirement for tumor outgrowth and metastasis, is regulated by pro- and antiangiogenic factors. Methylation-associated inactivation of the angiogenesis inhibitor thrombospondin-1 (TSP-1) has been observed recently in some adult tumors. To investigate the role of TSP-1 in pediatric cancer, we examined its pattern of expression and mechanism of regulation in neuroblastoma (NB). TSP-1 was silenced in a subset of undifferentiated, advanced-stage tumors and NB cell lines. In contrast, most localized tumors expressed this angiogenesis inhibitor, and a significant correlation between morphological evidence of neuroblast differentiation and TSP-1 expression was observed. Luciferase assays demonstrated the presence of nuclear factors required for TSP-1 transcription in both TSP-1 positive and-negative cell lines, but no correlation between TSP-1 promoter activity and the level of TSP-1 mRNA expression was seen. Our studies indicate that the transcriptional silencing of TSP-1 was caused by methylation. TSP-1 promoter methylation was detected in all of the NB cell lines lacking TSP-1 mRNA and in 37% of the NB clinical tumors analyzed. Furthermore, treatment with the demethylating agent, 5-Aza-2’-deoxycytidine (5-Aza-dC), restored TSP-1 expression in NB cell lines. Disrupting methylation with 5-Aza-dC also led to significant inhibition of NB in vivo and re-expression of TSP-1 in a subset of NB xenografts. These results suggest that 5-Aza-dC inhibits NB growth by augmenting the expression of TSP-1 along with other genes that suppress tumor growth. Demethylating agents may prove to be effective candidates for the treatment of children with NB.


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Monica Morrow, MD
Cancer Center Events

CONTINUING MEDICAL EDUCATION PROGRAMS
Throughout the year, the Robert H. Lurie Comprehensive Cancer Center of Northwestern University offers Continuing Medical Education (CME) programs on various cancer specialties. Below is a list of the programs for the remainder of 2004. For specific dates or more information about these programs, visit www.cancer.northwestern.edu or call the Cancer Center at (312) 695-1304.

NCCN Regional Guidelines Symposium: Colon, Rectal, Anal and Pancreatic Cancers
June 25, 2004

American Society of Clinical Oncology 2004 Review
July 30, 2004
Chair: William Gradishar, MD

7th Annual Oncology Nursing Conference
October 8, 2004

6th Annual Lynn Sage Breast Cancer Symposium
Oct. 28-31, 2004
Chairs: William Gradishar, MD, V. Craig Jordan, OBE, PhD, DSc, Monica Morrow, MD

COMMUNITY EVENTS/PATIENT PROGRAMS
The Cancer Center is committed to educating the public about cancer prevention and treatment. Many community events and patient programs are offered throughout the year. For more information about these programs, please visit www.cancer.northwestern.edu or call the Cancer Center Special Events Hotline at (312) 695-1304.

Cancer Survivors’ Celebration and Walk
June 6, 2004 (National Cancer Survivor’s Day)

Lynn Sage Breast Town Hall Meeting
September 12, 2004
The Robert H. Lurie Comprehensive Cancer Center of Northwestern University is the focus of cancer research, treatment and education at Northwestern University. The Cancer Center coordinates and integrates the University’s cancer and cancer-related activities and units scientists, clinicians and educators in the fight against cancer. The Cancer Center’s administrative offices and many of its basic science research activities are at Northwestern University’s Feinberg School of Medicine on the Chicago campus. Additional offices and basic science research labs are located on the Evanston campus. Clinical research is conducted at the Feinberg School of Medicine’s various affiliated teaching hospitals: Northwestern Memorial Hospital, Children’s Memorial Hospital, Evanston Northwestern Healthcare, the Rehabilitation Institute of Chicago and Veterans Administration Chicago Healthcare System.