



**GENETICS &
BIOLOGY OF
CHILDHOOD
CANCER
2009**

Traveling the road of childhood cancer, from cause to cure...

February 26–27, 2009

located at



HOTEL CONTESSA™

Suites on the Riverwalk

 GREEHEY CHILDREN'S
CANCER RESEARCH INSTITUTE
**UT HEALTH
SCIENCE CENTER™**
SAN ANTONIO

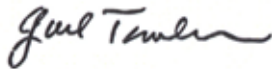
February 26, 2009

Dear Symposium Attendees:

Welcome to the Greehey Children's Cancer Research Institute's Scientific Symposium, "**Genetics and Biology of Childhood Cancer** 2009: *Traveling the road of childhood cancer from cause to cure.*"

This symposium brings together investigators from a variety of scientific perspectives with a common theme and goal – understanding childhood cancer and working towards a cure. Over the next two days, we hope you will experience exciting scientific discourse and will leave with fresh insights that may be applied to your own research. Perhaps some new collaborations will emerge as well.

We also hope that you will take some time to enjoy the San Antonio Riverwalk.



Sincerely,

Gail E. Tomlinson, M.D., Ph.D.

*Interim Director, Greehey Children's Cancer Research Institute
on behalf of the symposium organizing committee:*

Gail E. Tomlinson, MD, PhD, **Chair**

Danette Besancon
Alexander J. R. Bishop, DPhil
Leslie Burks, MA
William Chessher, MHA
Charles Keller, MD
Anthony Mendicino, CPA, CITP
Vivienne I. Rebel, MD, PhD
Zhi-Min Yuan, MD, MS, PhD

Thursday, February 26, 2009The General Session will be in the *Cypress Room* at the Contessa

7:30–10:00	Registration/Name Badge Distribution/ Poster Check-In	12:30–2:00	Lunch – Buffet included with registration fee
10:00–12:30	Session I – Genetics & Epidemiology Advances in genomic technology have led to increasing recognition of the role of predisposition in pediatric cancer. Ethnicity, family history and developmental anomalies work together to define cancer risks; recent advances in the genetic background of childhood cancers will be presented in this session.	2:00–4:30	Session II – Animal Models, Genes & Pathways: Fundamental properties of tumors, such as signaling pathways, cellular self-renewal and cell-microenvironment interactions, are being uncovered at a rapid rate using exciting approaches with genetic model organisms. Examples of these advances will be presented in this session.
10:00–10:20	Session Chair: Gail Tomlinson, MD, PhD	2:00–2:10	Session Chair: Vivienne Rebel, MD, PhD
10:25–10:45	Guest Speakers: Brad Pollock, PhD (UT Health Science Center) <i>“Cancer in children and adolescents in Texas: A unique population?”</i>	2:15–2:35	Guest Speakers: Scott Cameron, MD, PhD (The University of Texas Southwestern Medical Center at Dallas, TX) <i>“Discovery of developmental pathways altered in hematopoietic disease”</i>
10:50–11:10	Louise C. Strong, MD (The University of Texas M. D. Anderson Cancer Center, Houston, TX) <i>“Li Fraumeni Syndrome: Cancer risk and risk modifiers”</i>	2:40–3:00	Charles Roberts, MD, PhD (Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA) <i>“Rhabdoid tumors: Epigenetics, chromatin and cancer”</i>
11:15–11:35	Linda Scott, PhD (UT Health Science Center) <i>“JAK2, and the molecular pathogenesis of down syndrome-associated acute lymphoblastic leukemia”</i>	3:05–3:25	Vivienne Rebel, MD, PhD (UT Health Science Center) <i>“Myelodysplastic Syndrome: A view from the niche”</i>
11:40–12:00	Sharon Plon, MD, PhD (Baylor College of Medicine, Houston, TX) <i>“Identification of genetic susceptibility to childhood cancer through high throughput sequencing of genes in parallel”</i>	3:30–3:50	James Francis Amatruda, MD, PhD (The University of Texas Southwestern Medical Center at Dallas, TX) <i>“The BMP pathway and differentiation in germ cell tumors”</i>
12:05–12:30	Ray Stallings, PhD (Royal College of Surgeons and Our Lady’s Children’s Hospital, Dublin, Ireland) <i>“Genome-wide mapping of MYCN binding sites in the neuroblastoma genome”</i>	3:55–4:15	Mark A. Israel, MD (Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH) <i>“New insights into cellular metabolism: Implications for cancer therapy”</i>
		4:30–6:00	Poster viewing session with drinks and appetizers

Friday, February 27, 2009The General Session will be in the *Cypress Room* at the Contessa

7:30–8:00	Continental Breakfast	10:30–10:45	Break
8:00–10:30	<p>Session III – Genetic Instability & DNA Repair:</p> <p>The maintenance of genomic stability following DNA damage by DNA surveillance and repair mechanism is essential for cellular and organismal health. When these cellular defense mechanisms are absent, genomic instability and cancer predisposition are frequently observed. Further, considering that many cancers are treated by radiation and chemotherapeutics that are DNA damaging agents, understanding how the normal or cancer cell responds is of clinical relevance. By studying these processes we will develop novel insights into cancer etiology and progress towards better, more targeted therapies.</p> <p>Session Chair:</p> <p>Zhi-Min Yuan, MD, MS, PhD</p> <p>Guest Speakers:</p> <p>Albert J. Forance, Jr., MD (Georgetown University Medical Center, Washington, DC) <i>“Modulation of oncogenic and genotoxic stress responses by WIP1 phosphatase, the product of the PPM1D oncogene which is amplified in pediatric tumors including neuroblastoma and medulloblastoma”</i></p> <p>Matthew H. Porteus, MD, PhD (The University of Texas Southwestern Medical Center at Dallas, TX) <i>“DNA double-stranded breaks: A path towards causing cancer, curing cancer and curing genetic diseases”</i></p> <p>David J. Chen, PhD (The University of Texas Southwestern Medical Center at Dallas, TX) <i>“DNA-PKCS phosphorylation in response to DNA damage and in hematopoietic stem cell maintenance”</i></p> <p>Alexander J.R. Bishop, DPhil (UT Health Science Center) <i>“A conserved damage survival network and its implications in cancer treatment”</i></p>	10:45–12:15	<p>Session IV – 10–Minute presentations of selected posters, 5 minute Q&A:</p> <p>10:45 Jeffrey C. Murray: <i>“Childhood brain tumors might occur more frequently in twins: A single institution observation, 2003-2007”</i></p> <p>11:05 J. Saadi Imam: <i>“miRNAs involved in kidney development are disregulated in Wilms’ tumor”</i></p> <p>11:25 William E. Haskins: <i>“Proteomics of neoplastic (cancer) stem cells in children’s germ cell tumors”</i></p> <p>11:45 Samy Lewiz Habib: <i>“Mechanisms of DNA damage/repair in renal tumorigenesis”</i></p> <p>12:05 Jason M. Shohet: <i>“MDM2 Haploinsufficiency delays tumorigenesis in pTh-MYCN transgenic mice”</i></p>
8:00–8:10		12:30–2:00	Lunch–Buffet
8:15–8:40		2:00–4:30	<p>Session V – Disease Models & Preclinical Studies:</p> <p>Carefully designed xenograft and genetically-modified animal models now reveal interesting drug targets and give insight into mechanisms of therapeutic efficacy and resistance. By integrating basic science, <i>in vivo</i> studies and the NCI Pediatric Preclinical testing program, the development of novel treatment regimens can now be accelerated.</p> <p>Session Chair:</p> <p>Charles Keller, MD</p> <p>Guest Speakers:</p> <p>A. Thomas Look, MD (Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA) <i>“Activating mutations in the alk tyrosine kinase provide a therapeutic target in neuroblastoma”</i></p>

- 2:40–3:00 Rene Lawrence Galindo, MD, PhD
(The University of Texas Southwestern Medical Center
at Dallas, TX)
*“Drosophila muscle and that rhabdomyosarcoma
coma oncoprotein PAX-FKHR”*
- 3:05–3:25 Charles Keller, MD
(UT Health Science Center)
*“Muscling in on rhabdomyosarcoma origins
and therapies”*
- 3:30–3:50 C. Patrick Reynolds, MD, PhD
(Texas Tech University Health Science Center,
Lubbock, TX)
*“Retinoids in cancer therapy:
Size does matter!”*
- 3:55–4:15 Peter J. Houghton, PhD
(St. Jude Children’s Research Hospital, Memphis, TN)
*“The pediatric preclinical testing program.
results and challenges”*
- 4:30 **Wrap-up and Adjourn**

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The Greehey Children's Cancer Research Institute

History

The Children's Cancer Research Institute (CCRI) was created in 1999 by the 76th session of the Texas Legislature setting aside \$200 million from the settlement the state received in a class action lawsuit against big tobacco companies. That decision created the largest single cancer endowment in the nation's history, and provided the funding for The University of Texas Health Science at San Antonio to "establish, maintain and operate a children's cancer center." In February

2004, leaders including the director of the National Cancer Institute celebrated the dedication of the \$50 million research building and opened its doors for recruiting outstanding scientists.

In January, 2007, Valero Energy Corporation Chairman Bill Greehey announced that

his family foundation had contributed an unprecedented \$25 million to The University of Texas Health Science Center at San Antonio. The Greehey family gift is one of the single largest cash contributions in The University of Texas System's and San Antonio's history. To recognize the importance of the gift, Health Science Center officials announced that their North Campus will be renamed the Greehey Academic and Research Campus, and the Children's Cancer Research Institute became the Greehey Children's Cancer Research Institute.

Structure

The Greehey Children's Cancer Research Institute (GCCRI) is organized around several major programs and shared core facilities, and is composed of an interdisciplinary group of faculty spanning multiple departments and schools of the Health Science Center. The major programs include cancer genetics, experimental therapeutics and cancer control. Faculty members have been recruited from around the world, and bring their expertise to this collaborative matrix environment from many disciplines.

Purpose

The fundamental theme of the Greehey Children's Cancer Research Institute is to approach a complete understanding of the genetic and molecular mechanisms underlying pediatric cancers will lead to improvements with a positive

impact not just on childhood cancers but also cancer at all ages.

Our specific missions are to:

1. To advance scientific knowledge relevant to childhood cancer
2. To accelerate the translation of knowledge into novel therapies
3. To disseminate scientific knowledge relevant to childhood cancer

Location

The state-of-the-art research building is on the Greehey Academic and Research Campus, and contains 100,000 square feet of laboratory, office, and support space for principal investigator teams. The Greehey Academic and Research Campus also includes the School of Health Professions Building and the Robert F. McDermott Clinical Science Building. Construction is underway on the adjacent South Texas Research Facility.



Greehey Children's Cancer Research Institute
Photographer: Frank Ooms

The Greehey Children's Cancer Research Institute - Faculty

Christine Aguilar, MD, MPH
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Yidong Chen, PhD
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Ravi Dashnamoorthy, PhD
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Luiz Penalva, PhD
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Manjeet Rao, PhD
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Zhi-Min Yuan, MD, PhD
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Fuchun Zhou, DVM, PhD
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*Symposium Speaker

Invited Speaker Profiles

James Francis Amatruda, MD, PhD, is Assistant Professor of Pediatrics, Molecular Biology and Internal Medicine at The University of Texas Southwestern Medical Center at Dallas. He received his M.D. and Ph.D. degrees from Washington University in St. Louis, and completed his residency in Internal Medicine at the Brigham and Women's Hospital and a Hematology-Oncology fellowship at the Dana-Farber Cancer Institute. He joined the UT Southwestern faculty in 2005. His research interests are centered on childhood cancers including germ cell tumors and Ewing's Sarcoma, and the use of the zebrafish system to model the genetics of human cancers.

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Scott Cameron, MD, PhD, is the Children's Cancer Fund Scholar in Biomedical Research and an Assistant Professor of Pediatrics and Molecular Biology at UT Southwestern. He completed graduate studies in Dr. Michael Wigler's laboratory at Cold Spring Harbor Laboratory, medical training at MIT/Harvard Medical School, Children's Hospital and Dana-Farber Cancer Institute, and postdoctoral training in Dr. H. Robert Horvitz's laboratory at MIT. At UT Southwestern he is a pediatric hematologist-oncologist, and co-Chair of the Development and Cancer Scientific Program in the Simmons Comprehensive Cancer Center. His laboratory uses discovery and analysis of developmental pathways that regulate programmed cell death in *C. elegans* to suggest novel hypotheses about the etiologies of human disease.

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David J. Chen, PhD, is Director and Professor of Division of Molecular Radiation Biology, Department of Radiation Oncology at UT Southwestern Medical Center in Dallas, Texas. He received his PhD in Genetics from the University of Missouri and his post-doctoral work was completed at Los Alamos National Laboratory. His research is focused on the mechanisms by which cells recognize, respond to, and repair of ionizing radiation induced DSBs. Deficiencies in DNA-

damage signaling and repair pathways are fundamental to the etiology of most human cancers. Of the many types of DNA damage that occur within the cell, DNA double-strand breaks (DSBs) are particularly dangerous. DSBs are caused by both endogenous and exogenous threats, including ionizing radiation (IR). An inability to respond properly to DSBs or to repair them correctly can lead to mutation, genome instability, cell death and tumorigenesis. A cell's ability to repair damaged DNA is critical, not only for the prevention of malignancy, but also for the resistance of many tumors to current therapies. Understanding DNA repair processes and cellular radiation responses in general will therefore help to improve the efficiency of radiation treatment and to protect the normal tissues during radiation therapy. Dr. Chen's research is supported by NIH and NASA.

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Albert J. Fornace, Jr., MD, focuses on stress signaling pathways involved in the cellular responses to toxicant as well as oncogenic stresses. Scientific contributions include an early role in the development of sensitive assays to monitor DNA damage and repair in mammalian cells, and then the identification and characterization of mammalian stress response genes including the discovery of some of first mammalian DNA-damage inducible genes. The laboratory is probably best known for the discovery and cloning of the gadd (growth arrest and DNA damage inducible) genes and the finding that stress-induction of the Gadd45a gene is dependent on the tumor suppressor p53 and the ATM gene product. The Fornace Lab was the first to demonstrate regulation of a stress gene, Gadd45a, by p53 and the key role for the p53 signaling pathway in the maintenance of genomic stability. Continuing projects have focused on stress signaling and cellular responses to aberrant oncogene expression and genotoxic agents, which contribute to tumor development and also are used in cancer therapy. The laboratory has shown critical roles for the p38 MAP kinase family as a tumor suppressor and interplay between various key signaling pathways such as p53, MAP kinase, Rb, ATM, and others. These projects involve a variety of novel mouse models including gene disruption (knockout) and site-directed mutation (knockin) strategies with relevance to cancer and a variety of injury responses. Considering the complexity of genotoxic stress responses, another major focus is the development of functional genomic and metabolomic approaches

to monitor for stress response at the genome-wide level. Dr. Fornace was chief of the Gene Response Section in the Center for Cancer Research at NCI, and is currently a professor in Oncology and also Biochemistry and Molecular & Cellular Biology at Georgetown Univ., and an adjunct professor and director of the John B. Little Center for the Radiation Sciences and Environmental Health at Harvard School of Public Health. He holds the first Molecular Cancer Research Chair at the Lombardi Comprehensive Cancer Center at Georgetown. He received his MD from Jefferson Medical School and post-doctoral training at the NCI and Harvard.

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Rene L. Galindo, MD, PhD, is a pediatric pathologist and Assistant Professor in the Department of Pathology at the University of Texas Southwestern Medical Center at Dallas (UTSWMC). His clinical and research interests focus on pediatric soft tissue malignancies (sarcomas). His research lab is presently using both invertebrate and mammalian model systems to investigate the pathogenesis of the most common childhood sarcoma, rhabdomyosarcoma. In addition to his primary appointment, he also holds joint appointments in the Department of Molecular Biology and the UTSWMC Simmons Comprehensive Cancer Center.

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Peter J. Houghton, PhD, is a member of the Soft Tissue Sarcoma Scientific Committee in COG for 12 years, Chaired the Pharmacology Committee, Pediatric Brain Tumor Consortium, and is a member of the Phase I consortium Developmental Therapeutics Committee, and chairs the Biology subcommittee. His research interests are: IGF-I mediated survival pathways in malignant cells derived from childhood tumors and developmental therapeutics for childhood solid tumors.

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Mark Israel, MD, received his undergraduate education at Hamilton College (1968) and his medical training from Albert Einstein College of Medicine (1973). After an internship and a residency at the Children's Hospital Medical Center in Boston, he moved to the NIH to pursue research at the National Institute of Allergy and Infectious Diseases. In 1981 he completed his training in pediatric oncology in the Pediatric Branch of the National Cancer Institute, where he ultimately became head of the Molecular Genetics Section in 1984. In 1990 he moved to San Francisco as Professor of Neurological Surgery and Pediatrics to lead the Preuss Laboratory of Molecular Neuro-Oncology. From 1997 he was the Kathleen M. Plant Distinguished Professor. In 2001, Dr. Israel moved to Dartmouth Medical School as a Professor of Pediatrics and of Genetics and as Director of the Norris Cotton Cancer Center, an NCI-designated comprehensive cancer center.

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A. Thomas Look, MD, received his M.D. degree and post-graduate training in Pediatrics from the University of Michigan, in Ann Arbor, Michigan, and his fellowship training in pediatric oncology at St. Jude Children's Research Hospital in Memphis, Tennessee. He then accepted a faculty position at St. Jude, and remained on the faculty for 20 years, ultimately becoming the Chair of the Experimental Oncology Department at St. Jude Children's Research Hospital. In June of 1999, he joined the Dana-Farber Cancer Institute in Boston, Massachusetts as Vice-Chair for Research in Pediatric Oncology and Professor of Pediatrics at Harvard Medical School. Research in his laboratory focuses on the molecular pathogenesis of leukemia, and has resulted in the identification and functional analysis of several chimeric oncogenes activated by chromosomal translocation, including the discovery that E2A-HLF transcription factor acts through an evolutionarily conserved pathway to promote leukemia cell survival. His work in human T-cell acute lymphoblastic leukemia has led to the identification of five multistep mutational pathways and the discovery that NOTCH1 receptors are mutationally activated in the majority of patients with this disease. His recent work led to the first transgenic model of leukemia in the zebrafish, paving the way for chemical and genome-wide genetic modifier screens in a vertebrate disease model.

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Sharon E. Plon, MD, PhD, FACMG, holds two B.S. degrees from MIT and is a graduate of the Harvard University Medical Scientist Training Program with a PhD in Biophysics. She completed residency and fellowship training from the University of Washington under a Howard Hughes Medical Institute postdoctoral fellowship. She is board-certified in medical genetics. She was appointed to the BCM faculty in 1993 and is currently Associate Professor in Departments of Pediatrics, Molecular and Human Genetics and Human Genome Sequencing Center. Dr. Plon developed the clinical cancer genetics program at BCM which has been an NCI-funded site for the Cancer Genetics Network since 1998. Her laboratory research on genomic instability and cancer susceptibility genes has been continuously funded by NIH since coming to

BCM and she currently holds an R01 from the NHGRI and Innovation Grant from ALS Childhood Cancer foundation. Her leadership in cancer genetics includes being member and past chair of the Steering Committee of NHGRI Breast Cancer Information Core, chair of the Translational Working Group of the CGN, chair of the International Working Group on clinical interpretation of genetic variants, Cancer Genetics Editor of Genetics in Medicine and numerous invited talks.

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Matthew H Porteus, MD, PhD, is an Assistant Professor in Pediatrics and Biochemistry at UT Southwestern Medical Center. He received his bachelor's degree at Harvard, his MD/PhD from Stanford, and completed his pediatrics and pediatric hematology/oncology training at Boston Children's Hospital. Dr. Porteus joined the faculty at UT Southwestern in 2003. His research interests include using homologous recombination as an approach to gene therapy and understanding the metabolism of DNA double-strand breaks.

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C. Patrick Reynolds, MD, PhD, is the Associate Dean for Research and the Director of the Cancer Center for the Texas Tech University Health Sciences Center School of Medicine, Lubbock, Texas, and Director of the South Plains Oncology Consortium. Dr. Reynolds is Chair of the Neuroblastoma Developmental Therapeutics Sub-committee and member of the steering committees for neuroblastoma and translational research for the Children's Oncology Group. He is a special government employee with the FDA and a member of the Pediatric Subcommittee of the Oncologic

Drugs Advisory Committee for the FDA. He is a member of the New Approaches to Neuroblastoma Therapy (NANT) consortium Scientific Review Committee, a member of the Pediatric Oncology Task Force of the American Association for Cancer Research. He is a member of the Drug Discovery and Molecular Pharmacology Study Section for the National Cancer Institute and a member of the Best Pharmaceuticals for Children Act prioritization panel for the National Institute for Child Health and Development. Dr. Reynolds received his BA in Biology from The University of Texas at Austin, his MD from UT Southwestern Medical School in Dallas, TX, his PhD in Cell Biology from UT Austin, and his pediatrics training at the National Naval Medical Center. His postdoctoral fellowship was in cancer immunology at UT Southwestern Medical School, Dallas, TX.

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Charles Roberts, MD, PhD, an Assistant Professor and physician-scientist at Harvard Medical School, studies the role of epigenetics in cancer pathogenesis. He uses the Swi/Snf chromatin remodeling complex as a model as this complex contributes to epigenetic regulation via the control of nucleosome position. Subunits of the Swi/Snf complex are mutated in a variety of human cancers and targeted disruption in mice leads to the rapid onset of cancer in all mice. Dr. Roberts has also collaborated with members of the Broad Institute in the development of computational methods for comparing gene expression profiles across species.

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Raymond L. Stallings, PhD, a graduate of Texas A&M University and the University of Texas Health Science Center in Houston, is a recognized leader in the field of genetic organization of chromosomes. Dr. Stallings was a founding member of the Greehey Children's Cancer Research Institute and was Professor of Pediatrics at the University of Texas Health Science Center at San Antonio from 2004 to 2007. Prof. Stallings currently holds the Chair of Cancer Genetics at the Royal College of Surgeons in Ireland and is also the program leader of cancer genetics at the Children's Research Centre at Our Lady's Children's Hospital in Dublin.

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Abstracts

1 Proteomics of Neoplastic Germ Stem Cells in Children's Germ Cell Tumors

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Children's germ cell tumors, believed to arise from transformed primordial germ cells by an unknown mechanism, provide a unique model system for investigating neoplastic germ stem cells in their natural microenvironment. Two-dimensional gel electrophoresis was performed for four dysgerminomas and four childhood endodermal sinus tumors, resembling self-renewing and differentiating germ stem cells, respectively. Nine of nine hundred forty-one spots were differentially expressed with greater than a two-fold change in spot volume for at least three of four gels in each group. Two of nine spots had p-values for the t-test analysis of comparisons less than 0.001, while the remaining spots had p-values from 0.013 to 0.191. Capillary liquid chromatography-tandem mass spectrometry with protein database searching identified top-ranked proteins in all nine spots with 4.0 to 38% sequence coverage. APOA1, CRK and PDIA3 were up-regulated in childhood endodermal sinus tumors; TFG, TYMP, VCP, RBBP, FKBP4 and HSPA5 were up-regulated in dysgerminomas. The fold-changes observed by proteomics are in general agreement with transcriptomics results for the same specimens and characteristic genetic changes. Comparison of our results with published work suggests that the functions of FKBP4 and the glucocorticoid receptor in cancer germ cells merit further investigation.

2 Childhood brain tumors might occur more frequently in twins: A single institution observation, 2003–2007

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Introduction

Identical/monozygotic twinning occurs at a constant rate of

0.4% in the general population and fraternal twinning occurs at a variable rate of approximately 2–3%, depending on maternal age and geography. Although monozygotic twins appear to have a concordant risk of developing hematopoietic malignancy (e.g., both twins developing AML), several studies have suggested that twins overall develop less childhood cancer than singleton children. The reason is unknown. Large population studies from Sweden and the United States, examining all types of childhood cancer, have not noted a higher rate of childhood brain tumors in twins, nor a concordance risk (i.e., the twin developing a brain tumor after the proband twin is diagnosed).

Purpose

We have observed over the past years a seemingly increased frequency of children with newly diagnosed brain tumors being one of twins. We sought to formally review our patient database to determine the incidence of such twin scenarios.

Methods

Following IRB approval, we performed a review of our brain tumor registry and studied charts of patients who were one of twins, diagnosed from 2003–2007. Charts were abstracted for tumor histology, family/birth history and current status.

Findings

129 total new brain tumor diagnoses were identified over the 5 year review period. 10 cases (7.8%) were identified where the proband case was one of twins. 5 were fraternal twins, 4 were identical/monozygotic twins and 1 patient was of unknown twinning status. Ages ranged from 6 months to 12 years. There were 8 boys and 2 girls. Histology revealed: 4 W.H.O Grade I gliomas (pilocytic); 3 medulloblastomas (MB/PNET); 2 W.H.O. Grades II-III gliomas; and 1 germ cell tumor (GCT). There were no concordant cases (i.e., both twins having a brain tumor).

Conclusions

Our observation of a seemingly higher frequency of brain tumor patients being one of twins is curious, and has not been reported from large multi-institution registries. This finding warrants awareness and further cooperative group registry analysis in the future. With more knowledge about cancer genomics and environmental risk factors, and as more children are enrolled in cancer registries, the scenario of brain and other cancers occurring in twins will be better understood.

3 Investigation of the Synthetic Lethality of PARP1 and BRCA1

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BRCA¹ and BRCA² are widely believed to be involved in homologous recombination repair (HRR) and inherited mutations of these genes are associated with increased risk of breast and ovarian cancers. Interestingly, studies have shown that inhibition of Poly (ADP-ribose) polymerase (PARP1) is lethal BRCA¹ or BRCA² deficient cells. The suggested mechanism for this synthetic lethality is that the combined loss of single strand break repair and double strand break repair, caused by loss of PARP1 and BRCA^{1/2}, respectively, leads to accumulation of DNA damage and subsequently cell death. This hypothesis relies on the following assumptions:

1. Cells lacking PARP1 will be unable to repair single strand breaks, 2. DNA replication converts these single strand breaks to double strand breaks, which are substrates for HRR, and 3. This HRR is BRCA^{1/2} dependent. By first crossing *Parp1* and *Brca1* mutants onto the homozygous p^{un/un} background, we were able to investigate these assumptions *in vivo* using the eyebased p^{un} HRR assay. To investigate whether HRR is, indeed, increased in a PARP1 deficient background, we crossed mice heterozygous for a targeted null mutation of *Parp1* and found a highly significant increase in spontaneous HRR in *Parp1* null mice as compared to *Parp1* heterozygous and wild-type mice, supporting assumptions 1 and 2. To investigate the involvement of BRCA¹ in HRR *in vivo*, and because *Brca1* nullizygosity is embryonic lethal, we combined a *Brca1* null allele and a *Brca1* conditional allele, which has exon 11 flanked by LoxP sites. Tissue-specific Cre excision creates the *Brca1*Δ11 allele, and results in production of only the small isoform of BRCA1. We found that *Brca1*^{null/Δ11} HRR frequency was significantly decreased compared to *Brca1*^{+/+} and *Brca1*^{null/+}, indicating that full length BRCA1 is involved in spontaneous HRR *in vivo*, supporting assumption 3. Together, our studies of PARP1 and BRCA1 deficiency *in vivo* support the previously proposed mechanism for this synthetic lethality. In addition to these results, we found that *Brca1*^{+/Δ11} spontaneous HRR frequency was decreased compared to *Brca1*^{+/null}, indicating a possible dominant negative effect by the *Brca1*Δ11 allele. This unexpected result is of particular interest given that the BRCA1Δ11 isoform is highly expressed in human breast epithelial cells and in many breast tumors.

4 Validating Igf1-r as a therapeutic target in alveolar rhabdomyosarcoma

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Alveolar rhabdomyosarcoma is a highly malignant muscle cancer which occurs predominantly in children and young adults. Patients with alveolar rhabdomyosarcoma have a dismal prognosis because the cancer is highly metastatic and untreatable in the advanced stages. The majority of alveolar rhabdomyosarcoma cases have the 2;13 chromosomal translocation which results in the formation of a chimeric gene, Pax3:Fkx. The detailed molecular mechanisms underlying tumor growth and metastasis in alveolar rhabdomyosarcoma are still not clearly understood. However, our initial studies examining the gene expression profiles for rhabdomyosarcoma samples from patients enrolled in a national clinical study has identified the IGF (insulin-like growth factor) signaling axis as being upregulated in alveolar rhabdomyosarcoma and associated with decreased disease-free survival. To test whether the Igf1-r (insulin-like growth factor1 receptor) signaling pathway is a critical regulator of tumor initiation and progression in alveolar rhabdomyosarcoma, we have generated a conditional mouse tumor model that authentically recapitulates the progression of human alveolar rhabdomyosarcoma. To dissect the disease-specific role of Igf1-r signaling pathway in rhabdomyosarcoma, we performed functional testing *in vitro*. Quantitative RT-PCR performed with rhabdomyosarcoma tumor specimen from children as well as from our immuno-competent mouse model showed considerable increase in the mRNA expression of both IGF ligands and receptors. Western blot analysis of the primary tumors and metastatic tumors from the mouse model also showed a significant increase in the protein levels of Igf1-r compared to the normal muscle. Immunohistochemistry (IHC) of mouse rhabdomyosarcomas further showed high expression of Igf1-r in tumor cells but not in the surrounding tissues. Anchorage-dependent colony formation assays show that 5 μM of NVP-AEW541 (an Igf1-r inhibitor) completely inhibited the growth of the mouse primary tumor cell cultures [IC₅₀ = 1.5 μM]. Soft-agar colony formation assay using a mouse rhabdomyosarcoma cell line showed a 20-70 percent reduction in colony size and number of colonies with increasing concentration of NVP-AEW541. Initial results suggest that oral administration of NVP-AEW541 inhibits tumor growth *in vivo*. Taken together, these data strongly support the involvement of IGF signaling in rhabdomyosarcoma progression and suggest a strong utility of the mouse model for testing of Igf1-r inhibitors for rhabdomyosarcoma.

5 The Effect of Genetic Background on Drug Response in Alveolar and Embryonal Rhabdomyosarcoma (RMS).

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RMS is the most common childhood soft-tissue sarcoma. RMS can be divided into two major subtypes: embryonal RMS (ERMS) and alveolar RMS (ARMS). Whereas, the majority of ARMS tumors share a common genetic translocation that results in the fusion of either Pax3 or Pax7 to the Fkhr gene, mutation of the Ptc1 (patched 1) gene is frequent in ERMS. These genetic mutations are often accompanied by mutations in the tumor suppressor gene, p53. These genetic mutations have been reproduced in genetically-engineered mice strains that express the mutated proteins in a time- and tissue-specific manner. These mice develop ERMS or ARMS in vivo and exhibit rapid progression of the disease. As a set of screening tools for preclinical trials in these mouse models, four cell lines isolated from primary tumors in mice with different genetic mutations were used to test the effect of genetic background on the efficacy of potential RMS drugs. The effect of four different compounds on the viability of these cell lines was tested in vitro. A conotoxin blocks the nicotinic acetylcholine gamma subunit, a cell surface receptor expressed in RMS, but not in normal muscle [Pubmed 18567854]. Treatment of four different RMS cell lines with a conotoxin resulted in no change in cell viability, most probably due to low levels of expression of the receptor. The potent mitogen, IGF-I, is strongly correlated with decreased disease-free survival in RMS patients [Pubmed 16261596]. NVP-AEW541 is a small molecule inhibitor that blocks the mitogenic effect of IGF-I. Treatment with NVP-AEW541 resulted in decreased cell viability in all cell lines, although the magnitude of the decrease differed depending upon whether the cell line was representative of ARMS or ERMS, with the ARMS cell lines exhibiting greater decreases in cell viability than the ERMS cell lines. Tumor cells depend primarily upon glycolysis, rather than oxidative phosphorylation, to support basic energy requirements. 2-DG (2-deoxy-D-glucose), an inhibitor of glycolysis, has recently been shown to induce apoptosis in two breast cancer cell lines and to increase the efficacy of adriamycin and paclitaxel in human osteosarcoma and non-small cell lung cancers in vivo [Pubmed 18445520]. Treatment of the RMS cell lines with 2-DG resulted in decreased cell viability in all cell lines. Interestingly, the changes in cell viability appeared to be related to the genetic mutations present in the cell line rather than the type of RMS cell line: cells with the Ptc1 mutation appeared to be more resistant to decreases in cell viability than cells with the Pax3:Fkhr fusion mutation. 3-GPA (3-guanidino-

propionic acid) decreases the level of phosphocreatine, thus depriving the muscle cell of its energy reservoir for oxidative phosphorylation [Pubmed 4814337]. Treatment with 3-GPA decreased cell viability in all cell lines to a similar extent, suggesting that basal levels of oxidative phosphorylation are similar in these cells regardless of the type of RMS or the genetic mutation. We hypothesized that combining 2-DG and 3-GPA would further deplete both the energy reserves of the RMS cells and result in decreased cell viability greater than either treatment alone. Surprisingly, the cell line expressing the Ptc1 mutation did not exhibit a further decrease in cell viability when treated with both 2-DG and 3-GPA. All other cell lines exhibited an additive effect of decreased viability when treated with both 2-DG and 3-GPA. These data suggest that the response of ARMS and ERMS to potential therapies is dependent upon both the type of RMS and the genetic mutations present in the tumor.

6 Establishment and Characterization of a Cancer Cell Line Derived from an Aggressive Childhood Liver Tumor

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Background

Hepatoblastoma is a rare malignancy of childhood. The scarcity of adequate cell models has limited our understanding of this tumor. Here we describe and characterize a new human liver tumor cell line, Hep293TT, derived from an aggressive childhood hepatoblastoma.

Procedures

Hep293TT cells were established using primary tumor tissues from a 5-year-old Caucasian female child. This cell line has been maintained for more than 30 months and over 20 subcultures, and was characterized by histopathology, ELISA,

genotype, cytogenetics, CGH array, Immunohistochemistry and molecular sequence analyses.

Results

Cells were confirmed to originate from parental tumor cells, secrete α -fetoprotein, and express hepatic markers and β -catenin. Hep293TT cells were able to form colonies in soft agar. Tumorigenicity was demonstrated by induction of solid tumors after subrenal capsule injection in immunodeficient mice. Hep293TT cells demonstrated a highly aneuploid karyotype, and a whole genome CGH analysis revealed chromosomal imbalances in every chromosome. Allelotype analysis demonstrated loss of alleles at distal 11p15.5 as is typical of embryonal tumors. Both Hep293TT cells and the primary tumor contain a deletion of 351 nucleotides in the β -catenin, as has been seen in other hepatoblastoma tumors. The cell line expressed β -catenin protein in both full-length and partially deleted forms. Gene sequencing revealed no mutation in the APC, MYH, MLH1, or MSH2 genes. The cell line expressed NOTCH2 protein, a marker for hepatoblasts.

Conclusion

This cell line is a valuable resource for the study of childhood liver cancer and may potentially provide a tool in the development of new agents.

7 Poster Withdrawn

8 Large-scale analysis of human protein and mRNA expression levels and their association with translation regulation

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Objectives

Translation is an essential part of eukaryotic gene expression regulation, which enables the dynamics of cellular systems and their responses to external and internal stimuli. Abnormal translation is a well-recognized characteristic of tumor cells and a potential target for therapy. While most of the studies have focused on individual regulators and specific targets, we currently lack system-wide analyses to evaluate the impact of translation regulation on tumorigenesis. We integrate proteomics and transcriptomics data for >1,000 human genes to analyze sequence-encoded correlates of translation levels.

Methods, and Results

Performing microarray and quantitative shotgun proteomics experiments in Daoy medulloblastoma cell lysate, we obtained high-confidence measurements of mRNA and protein expression levels for 1,025 human genes. We derive a

measure of 'translation efficiency', i.e. the combined outcomes of steady-state translation and protein degradation, by examining protein expression levels while accounting for the variation introduced by mRNA expression. We compare the characteristics of translation efficiency to 'transcription efficiency', i.e. the combined outcome of transcription and mRNA stability. For validation, we compare these data to datasets from complementary experimental techniques, and we obtain highly favorable results.

In the analysis we focus on a set of sequence features that are suspected to play dominant roles in translation regulation: length and nucleotide composition of the coding sequence and untranslated regions (UTRs), composition of the translation initiation site, presence of upstream open reading frames (uORFs), putative target sites of miRNAs, codon usage, and amino acid composition. We find that translation efficiency is mostly governed by the lengths of both coding sequence and the UTRs, the amino acid composition of the protein and the existence of putative uORFs in the 5'UTR. Amino acid composition, uORFs and putative regulation by miRNAs are characteristics with the largest difference in their effect on translation efficiency, as compared to transcription. We identify genes with strong evidence for uORFs repressing their translation (BRD2, ZNF462), and also a putative target of regulation by miR-181.

Conclusions

We provide one of the first large-scale measurements of protein and matching mRNA concentrations in human medulloblastoma cells. Tumorigenesis is strongly regulated at the level of translation, and we identify several sequence characteristics that govern global translation levels; and some of the characteristics are independent of transcription. We demonstrate that a large-scale study can provide novel strong candidates for known regulatory mechanisms, which can now be examined in more detail for their potential as targets of cancer treatment at the level of translation.

9 Pediatric Preclinical Testing Initiative

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Background

The Pediatric Preclinical Testing Initiative (PPTI) at GCCRI was established in August 2008 with the mission of identifying novel targeted therapies for childhood cancer using genetically-engineered mouse models.

Objective

The PPTI will work with the Cancer Therapy Evaluation Program (CTEP) at the National Cancer Institute to discover new, molecularly-targeted therapies that are more effective and less toxic than traditional chemotherapy. PPTI is the first implementation of genetically-engineered mice in the CTEP Pediatric Preclinical Testing Program. Each sponsorship (\$8,538) will test a specific drug. The PPTI will provide tangible results in 6–12 months in a written one page report.

Design/Method

PPTI models include alveolar (ARMS) and embryonal (ERMS) rhabdomyosarcoma and medulloblastoma. The ARMS model re-creates mutations known to occur in this muscle cancer of children by simultaneously activating Pax3:Fkhr and inactivating CDKN2A or p53. Mice develop tumors that are as aggressive as advanced stage human alveolar rhabdomyosarcoma. The ERMS mouse model is generated by deleting the Sonic Hedgehog Receptor and the p53 gene in muscle stem cells. The medulloblastoma model is engineered by deleting the Sonic Hedgehog Receptor and the p53 gene in the juvenile cerebellum.

Results

As an example, the prototypic receptor tyrosine kinase inhibitor, Imatinib was tested in the ARMS model. A dose of 50 mg/kg/day was administered by oral gavage for 2–4 weeks (cohort 13+ mice). Regression was seen in 8/10 animals; stable disease in 2/10 animals; resistance in 3/10 animals. Representative results are shown below (nb:generally cohorts will use 15–30 treated mice).

Conclusion

Imatinib treatment resulted in regression or stable disease in all animals. Larger tumors typically had better responses than smaller ones. Small tumors tended to evolve resistance despite an initial response. The goal of future studies is to prevent resistance from emerging. Similar studies can be conducted by request from academic laboratories or sponsoring foundations in any of the 3 models.

10 Prediction of interologues between five species and its utility in the analysis of Genomic Data

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A protein-protein interactome is a collection of interactions between proteins in a single organism; the conservation of any one interaction between orthologues across spe-

cies is known as an interologue. Since our knowledge of all protein-protein interaction for any one species is incomplete, we have taken a comparative biology approach to predict interactions in five species (human, mouse, fruitfly, nematode, budding yeast) using orthologue analysis, and we have determined the connectivity of these resulting networks. We have shown that some of these predictions are indeed observable in coimmunoprecipitation experiments conducted in fruitfly tissue culture cells. We have also assigned a confidence score to each predicted interaction based on conservation across species, and we will be providing access to this data, searchable by species. Two examples of how these improved interactomes can be used are: 1. analysis of the relationships of proteins with roles in diseases such as cancer, and 2. analysis of genomic-scale data, such as transcription data from microarray experiments.

11 A comprehensive *in silico* expression analysis of RNA binding proteins in normal and tumor tissue; identification of potential players in tumor formation

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Abstract

RNA binding proteins (RBPs) are involved in several post-transcriptional stages of gene expression and dictate the quality and quantity of the cellular proteome. When aberrantly expressed, they can lead to disease states as well as cancers. A basic requirement to understand their role in normal tissue development and cancer is the build of a comprehensive gene expression maps. In this direction, we generated a list with 383 human RBPs based on the NCBI and EMSEMBL databases. SAGE and MPSS were then used to verify their levels of expression in normal tissues while SAGE

and microarray datasets were used to perform comparisons between normal and tumor tissues. As main outcomes of our studies, we identified clusters of co-expressed or co-regulated genes that could act together in the development and maintenance of specific tissues; we also obtained a high confidence list of RBPs aberrantly expressed in several tumor types. This later list contains potential candidates to be explored as diagnostic and prognostic markers as well as putative targets for cancer therapy approaches.

12 Kaposi's Sarcoma-Associated Herpesvirus Disrupts Adherens Junctions and Increases Endothelial Permeability by Inducing Degradation of VE-Cadherin

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Kaposi's sarcoma (KS) is a vascular tumor of proliferative endothelial cells caused by KS-associated herpesvirus (KSHV) infection. Aberrant vascular permeability is a hallmark of KS manifested as multifocal edematous skin and visceral lesions with dysregulated angiogenesis and vast inflammatory infiltrations. In this study, we showed that KSHV infection increased the permeability of confluent endothelial monolayers to serum albumin, blood-derived cells, KSHV-infected cells, and KSHV virions. KSHV-induced permeability was associated with the disruption of adherens junctions and the degradation of vascular endothelial cadherin (VE-cadherin) protein. Both the inactivation of KSHV virions by UV irradiation and the blockage of de novo protein synthesis with cycloheximide failed to reverse the KSHV-induced disruption of adherens junctions. However, soluble heparin that blocked KSHV entry into cells completely inhibited KSHV-induced permeability. Furthermore, the KSHV-induced degradation of VE-cadherin was dose dependent on the internalized virus particles. Together, these results indicate that KSHV infection induces vascular permeability by inducing VE-cadherin degradation during virus entry into cells. KSHV-induced aberrant vascular permeability could facilitate virus spread, promote inflammation and angiogenesis, and contribute to the pathogenesis of KSHV-induced malignancies.

13 Role of mitochondrial DNA mutations in the progression of cancer.

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Mitochondrial alteration has been long proposed to play a major role in tumorigenesis, and mitochondrial DNA (mtDNA) mutations were found in a variety of cancer cells. Still, it is unclear that how these mutations contribute in the progression of cancer. In this study we utilized human cell lines carrying a hetero-/homoplasmic frame-shift mutation at NADH dehydrogenase (respiratory complex I) subunit 5 gene (ND5) which was also identified in a human colorectal cancer line. We found that with increasing mutant ND5 mtDNA content, respiratory functions such as oxygen consumption and ATP generation through oxidative phosphorylation declined progressively, while lactate production and dependence on glucose increased. Interestingly, the reactive oxygen species (ROS) levels and apoptosis exhibited antagonistic pleiotropy associated with mitochondrial defects. The anchorage dependence phenotype and tumor-forming capacity of cells carrying wild-type and mutant mtDNA were tested *in vitro* (soft agar assay) as well as *in vivo* (nude mice assay). Surprisingly, the cell line carrying the heteroplasmic ND5 mtDNA mutation showed significantly enhanced tumor growth compared to its homoplasmic form. Similar pattern was obtained from the analysis of a series of mouse cell lines carrying a nonsense mutation at ND5 gene. In conclusion, mtDNA mutations might play an important role in early stage of cancer development, possibly through alteration of ROS generation and apoptosis.

14 C-Terminal fragment of transforming growth factor beta-induced (TGFBI) protein is required for apoptosis in human osteosarcoma cells.

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Abstract

The purpose of this study was to determine whether an apoptotic signal resides in the C-terminal portion of the 68 kDa extracellular matrix protein transforming growth factor beta induced (TGFBI). *In vitro* studies have implicated TGFBI in cell adhesion, tumor progression, and angiogenesis.

In vivo and in vitro, the C-terminal portion of TGFBI is fragmented, yielding a truncated TGFBI of approximately 62 kDa. The protein sequence of the fragmented portion includes EPDIM and RGD integrin-ligand peptides. When fragmentation of C-terminal portions is blocked, apoptosis is low, even when the protein is overexpressed. If fragmentation occurs, apoptosis is observed. Whether full-length TGFBI or integrin-ligand peptides released from its C-terminus is necessary for apoptosis remains equivocal. More importantly, the exact portion of the C-terminus that conveys the pro-apoptotic property of TGFBI is uncertain. It is reportedly within the final 166 amino acids. Here we used MG-63 osteosarcoma cells and C-terminally-truncated TGFBI in add-back and overexpression experiments in order to determine if the apoptotic property is dependent upon the final 69 amino acids containing the EPDIM and RGD sequences. With MG-63 osteosarcoma cells, transforming growth factor (TGF)- β 1 treatment increased expression of TGFBI over 72 hours ($p < 0.001$). At this time point, apoptosis was significantly increased ($p < 0.001$) and was prevented by an anti-TGFBI polyclonal antibody ($p < 0.05$). Overexpression of TGFBI by transient transfection produced a 2-fold increase in apoptosis ($p < 0.01$). Exogenous purified TGFBI at concentrations of 37 to 150 nM produced a dose dependent increase in apoptosis ($p < 0.001$). Mass spectrometric analysis of TGFBI isolated from conditioned medium of MG-63 cells treated with TGF- β 1 revealed truncated forms of TGFBI that lacked integrin-ligand sequences in the C-terminus. Recombinant TGFBI truncated, similarly, at amino acid 614 failed to induce apoptosis. A recombinant fragment encoding the final 69 amino acids of the TGFBI C-terminus produced significant apoptosis in MG-63 and Saos-2 osteosarcoma cells. The apoptosis level of MG-63 cells was comparable to that induced by TGF- β 1 upregulation of MG-63 endogenous TGFBI. Mutation of EPDIM, but not RGD, diminished the apoptotic action of TGFBI, implicating the EPDIM integrin-binding sequence in the apoptotic pathway. We conclude that the pro-apoptotic actions are dependent on the C-terminus most likely to interact with integrins.

15 A conditional mouse model for assessing BLM in homologous recombination

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Bloom's Syndrome (BS) is a rare, autosomal recessive disorder that results in an increased propensity of various cancers. The gene mutated in BS, BLM is one of a five-member family of RecQ helicases found in humans. In addition to cancer

predisposition and early onset, clinical features of BS patients include dwarfing, facial abnormalities and fertility complications. Clinical diagnosis of BS is through identification of increased levels of sister chromatid exchange (SCE). Along with elevated SCE, cellular phenotypes of BS patient's cells include symmetric multiradial rearrangements and chromosomal breaks, of which results in chromosomal instability and cancer promotion.

Although the exact function of BLM is still unresolved, the cellular phenotype of elevated SCE suggests a role in homologous recombination (HR) and DNA replication. In vitro experiments indicate that BLM activity is structure-specific, and that this action is anti-recombinogenic. Studies to define this role in vivo have had limited success due to BLM's requirement in mouse development. To begin to assess the role of BLM in HR in vivo, we have utilized a conditional BLM mouse model and the in vivo pun eye-spot assay to detect HR.

In addition to its anti-recombinogenic role, BLM also undergoes posttranslational modifications following various types of DNA damage. Particularly of interest is the phosphorylation of Thr99 and Thr122 by the gene product responsible for the disease ataxia telangiectasia (ATM) following ionizing radiation (IR) exposure. We will also use this model to understand the role of BLM in IR-induced HR repair.

Our preliminary data confirms BLM involvement in HR. Mice deficient in BLM have an increased frequency of HR following endogenous DNA damage compared to wild-type. Additionally, mice heterozygous for BLM also have a moderate increase in HR frequency following endogenous DNA damage compared to wild-type mice, and this dosage effect is consistent with previous findings.

16 MDM2 Haploinsufficiency Delays Tumorigenesis in pTh-MYCN Transgenic Mice

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Suppression of wild type p53 mediated apoptosis is a requirement for MYC oncogene driven tumorigenesis. The major inhibitor of p53 function, MDM2, is a direct transcriptional target of both MYCN and MYC. We have previously shown that neuroblastoma tumors are exceptionally sensitive to MDM2 inhibition with the small molecule inhibitor Nutlin-3a. We therefore hypothesized that MDM2 plays a critical role in MYCN driven tumorigenesis. To test this hypothesis we

generated a novel compound transgenic mouse model of neuroblastoma by crossing an *Mdm2*^{+/-} haplo-insufficient allele into the tyrosine hydroxylase targeted MYCN mouse model (pTH-MYCN) to compare tumorigenesis and molecular phenotypes of *Mdm2* haplo-insufficient and wild type neuroblastoma tumors.

Method

We backcrossed *MDM2*^{+/-} mice 8 generations into the SVJ/129 background and crossed these with pTH-MYCN mice to generate *MDM2*^{+/-} / *MYCN*^{+/+} compound transgenics. Longitudinal observations were then performed and tumor size, incidence and rates of onset calculated. We also performed genetic and biochemical analysis of the resulting tumors.

Results

In addition to dramatic changes in tumor latency and incidence, we found as predicted, increased baseline levels of p53 and its transcription target p21CIP1 as well as reduced *Mdm2* protein levels. An unexpected observation was that majority of *Mdm2* haplo-insufficient tumors had no detectable *Arf* protein due to epigenetically silencing of the P19ARF/ink4a locus.

Conclusions

We demonstrate with a novel in vivo model of neuroblastoma that haploinsufficiency of *Mdm2* has dramatic effects on tumorigenesis in pTH-MYCN mice. We propose this is due to altered regulation of p53-mediated tumor suppressor functions in MYCN expressing neuroblasts. Tumor development in this context likely requires additional time to circumvent this activity through genetic or epigenetic alterations in the genes regulating p53 such as ARF. Our data suggest an important role for MDM2 in raising the threshold for p53 mediated apoptosis and lend further support to therapeutic strategies to reactivate p53 in neuroblastoma.

17 Loss of CREB binding protein alters the regulatory properties of the hematopoietic stem cell niche.

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throughout life through both self-renewal and differentiation. Two possible HSC niches that can influence HSC fate have been identified in the bone marrow, an osteoblast niche and a vascular (endothelial) niche. The osteoblast niche is a protective niche that keeps the HSCs in a quiescent state while the vascular niche is thought to be an active niche in which HSCs are recruited for proliferation, mobilization and differentiation. We previously showed that aging *CBP*^{+/-} mice have a defective HSC pool and increased myeloid cell production; this was concluded to be, in part, a result of a cell-autonomous defect. However, HSCs are regulated by both cell-autonomous signals and interactions with their stem cell niche. Therefore, we investigated whether the loss of CBP in the HSC microenvironment could contribute to the hematopoietic phenotype of *CBP*^{+/-} mice. Initially, we assessed if the main cellular components of the HSC niches, osteoblasts and endothelial cells, were altered in *CBP*^{+/-} mice by histology and colony-forming unit – fibroblast (CFU-F) assays. From histological analysis, we found that *CBP*^{+/-} mice have less trabecular bone and increased sinusoids. In support of these results, the frequency of osteoblast precursor cells measured by CFU-F assay was also decreased in *CBP*^{+/-} mice. Thus, these results support the notion that the HSC niches in the *CBP*^{+/-} mice are altered. To investigate whether the loss of CBP in the HSC microenvironment could influence HSC regulation, we used in vitro co-cultures of HSCs and stroma cells and in vivo bone marrow transplantation assays. We found that a *CBP*^{+/-} microenvironment lacks the ability to properly maintain self-renewal of wildtype HSCs; instead it stimulates myeloid cell differentiation. Thus, the regulatory properties of the HSC niches are altered by the loss of CBP in the microenvironment. Lastly, a candidate approach and comparative microarray analysis were used to elucidate possible mechanisms by which loss of CBP in the microenvironment influences HSC regulation. Together the results of these studies pointed to two main mechanisms by which the loss of CBP effects microenvironment mediated HSC regulation 1) *Mmp9* related alterations and 2) an altered endothelial cell populations. Both of these are known to play an important role in microenvironment mediated HSC regulation. Thus, this model provides a unique opportunity to further elucidate the mechanisms of microenvironment mediated regulation of HSCs that we are currently pursuing.

Hematopoietic stem cells (HSCs) are responsible for the maintenance and repair of the hematopoietic system

18 Mechanisms of DNA Damage/Repair in Renal Tumorigenesis: From cell culture, animal model to patients with Tuberous Sclerosis Complex

Samy Lewiz Habib

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The tuberous sclerosis complex (TSC) is caused by defects in one of two tumor suppressor genes, TSC-1 or TSC-2. TSC-2 gene encodes tuberin, a protein involved in the pathogenesis of kidney tumors, both angiomyolipomas and renal cell carcinomas. Loss of heterozygosity at the 8-oxoG-DNA glycosylase (OGG1) allele is found in human kidney clear cell carcinoma identifying loss of OGG1 function as a possible contributor to tumorigenesis in the kidney. We show recently that tuberin regulates OGG1 through the transcription factor NF-YA in cultured cells. However, we found that tuberin haploinsufficiency is associated with the loss of OGG1 and accumulation of oxidative DNA damage, 8-oxodG in rat kidney tumor. In the current study, the effect of tuberin-deficiency on OGG1 protein and mRNA levels as well as on 8-oxodG levels in kidney tumor from patients with TSC was determined. In addition, the phosphorylation level of downstream targets of mTOR, S6K in kidney tumor tissue from TSC patients was evaluated. Significant increase in tuberin phosphorylation and decrease in total tuberin expression were detected in kidney tumor from TSC patients compared to control kidney tissue. The increase in tuberin phosphorylation and the decrease tuberin expression are associated with decreased in OGG1 protein and mRNA levels in tumor tissue compared to control kidney tissue. The decrease OGG1 expression is also associated with significant decreased in the transcription factor, NF-YA expression in tumor tissue compared to control tissue. In addition, the levels of 8-oxodG are 4-fold higher in tumor tissue compared to control tissue. The significant increase in tuberin phosphorylation is also associated with increased in S6K phosphorylation in tumor tissue compared to control tissue. These data indicate that tuberin deficiency enhances mTOR activation by phosphorylation of S6K and downregulation of protein and mRNA expression of OGG1 resulted in accumulation of oxidized DNA in kidney tumor of TSC patients. These results define a novel mechanism of the regulation of DNA damage/repair in renal tumorigenesis in TSC patients.

19 P53 Suppression of developmental homologous recombination and tumorigenesis

Bijal Karia, Alexander J.R. Bishop

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Genomic instability is a naturally occurring event during early development that can be observed by measuring homologous recombination repair (HRR) frequency. In utero exposure to damaging agents can increase levels of HRR and absence of tumor suppressor gene activity results in increased genomic instability both lead to increased cancer predisposition. In particular, the tumor suppressor gene p53, is mutated in at least half of all human cancers. We have shown that p53 normally suppresses spontaneous HRR during early development, but the mechanism by which it does so is unclear. There has been some evidence of p53 control of base and nucleotide excision DNA repair processes (BER and NER, respectively), however this modulation by p53 is still poorly understood. We hypothesize that developmental genomic instability, in absence of a caretaker gene like p53, is important in tumorigenesis. We propose that p53 stimulates BER and NER to repair low levels of endogenous DNA damage during development, but a dysfunction of this leads to repair by HRR. To understand the functionality by which p53 modulates HRR frequency in vivo, we determined the capability of two mutant mouse models of p53 to suppress HRR and are attempting to correlate this with tumorigenesis. The p53 R172P mutant mice retain limited transcriptional functionality (regulating cell cycle genes but not apoptotic genes) while the p53 R172H mutant mice lack all transcriptional activity but retain some protein: protein interaction capability. Using the in vivo pun assay to measure HRR frequency, we show significant increased HRR frequency in the p53 R172H mutant versus the p53 R172P mutant mice. This suggests that the retention of transcriptional capability of cell cycle genes and/or protein:protein interactions and not apoptotic genes, may be responsible for the suppression of HRR frequency. p53 nullizygous mice are viable but have a strong cancer predisposition mainly T-cell lymphomas within six months of age, however the origin of these tumors is still unknown. To ascertain the importance of early genomic instability and tumorigenesis, we use a conditional mouse model that permits reconstitution of a functional p53 allele upon injection with tamoxifen, to determine whether the absence of p53 during embryonic or postnatal develop-

ment has an effect on tumor latency and spectrum. p53 is involved in controlling genomic instability, at least as measured by HRR, during early development. These studies have an important implication in genomic instability and correlation with cancer.

20 KSHV Infection and Risk Factors in the HIV Population from South Texas

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Kaposi's sarcoma (KS) is a vascular neoplasm of endothelial cells caused by infection of KS-associated herpesvirus (KSHV). While KSHV/HIV co-infection has a synergistic effect in KS progression, the mechanisms associated with these interactions are not clearly understood. We hypothesize that HIV infection could affect clinical outcomes of KS by modulating KSHV replication and the host immune response. Our previous studies have shown that KSHV epidemiology in South Texas is distinct, with a higher KSHV seroprevalence in blood donors than the rest of the US. However, risk factors for KSHV infection in the HIV-infected population in South Texas remain undefined. In this study, we examined KSHV seroprevalence and risk factors for KSHV infection in HIV-infected subjects. KSHV serological analysis showed an increased KSHV seroprevalence (41%) in the HIV+KS- patients as compared to that of HIV-KS- controls (14%). AIDS-KS patients were 100% KSHV seropositive by both LANA and ORF65 assays. CD4+ T cell counts were significantly decreased in HIV+KSHV+ subjects with detectable ORF65 lytic antibodies compared to HIV+KSHV- subjects ($p=0.0001$). However, similar CD4+ T cell counts were observed in HIV+KSHV+ subjects with detectable LANA antibodies compared to HIV+KSHV- subjects ($p=0.5919$). HIV+KSHV+ subjects positive for ORF65 antibodies had significantly higher HIV viral loads ($p=0.0431$) and a longer length of HIV infection ($p=0.0537$) compared to HIV+KSHV- subjects. HIV+KSHV+ subjects with <200 CD4+ T cell counts had

higher KSHV seroprevalence (OR=1.92, 95% CI: 0.94-3.89, $p=0.07$) and higher antibody titers (median O.D. 0.48 vs 0.32; $p=0.0105$). Regression analysis also showed a significant correlation between KSHV antibody titers and CD4+ T cell counts ($p=0.0000$) but not with CD8+ T cell counts. These results indicate that impaired immune function might modulate KSHV infection and reactivation and KS.

21 A Glu-Asp Acidic Motif of The DSS1 Binds to Human Proteasome 19S Rpn3/S3 and Is Required for the Maintenance of Proteasome Interaction and Ubiquitin-mediated Protein Degradation

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Evidence supports the proposal that keratinocyte stem cells (KSCs) are a major target in skin tumorigenesis. Use of integrin alpha6briCD34bri KSCs to identify and characterize TPA-inducible genes in Tg.AC mouse model not only allows for better phenotypic definition of the target cell population, but also potentially defines gene expression changes in carcinogen-susceptible cells long thought to reside in the hair follicle bulge region. We used a combination of fluorescence-activated cell sorting (FACS) and cDNA microarray to identify 11 genes whose expression changed significantly in the tumor promoter TPA-treated integrin alpha6briCD34bri KSCs. DSS1 was one of TPA up-regulated genes and identified as a novel gene expressed in KSCs, with possible involvement in early skin tumorigenesis. DSS1 was originally identified in patients with the inherited heterogeneous limb developmental disorder called split hand/split foot malformation type 1. As compared to adjacent normal tissue, DSS1 expression was markedly elevated in TPA-induced skin hyperplasia, papillomas, spindle cell tumors, and squamous cell carcinomas in mice. Consistent with DSS1 expression localized to the regions of rapid cell growth, constitutive expression of mouse DSS1 can stimulate focus-formation and cell proliferation in preneoplastic JB6 Cl 41-5a epidermal cells. The mechanism by which DSS1 exerts its biological effects and causes cancers is currently unknown, but it is possible

that DSS1 participates in the ubiquitin-proteasome system (UPS). Studies further identify a specific function of the DSS1 in proteasome regulation by a direct interaction with the proteasome complex via the Rpn3/S3 subunit of the 19S regulatory particle (RP). Molecular anatomy of DSS1 revealed a Glu/Asp-rich acidic domain motif or Rpn3/S3-interacting motif (R3IM). Interestingly, this interaction is highly conserved throughout evolution from nematodes to humans. Deletion or substitution of the DSS1 Glu or Asp at R3IM prevents the Rpn3/S3-mediated DSS1 binding to the proteasome complex, suggesting the importance of the negative charges in the regulation of the proteasome stability and the binding capacity of ubiquitylated substrates. Complementing our biochemical data, our functional studies using RNAi to knockdown endogenous DSS1 in HeLa cells showed an increase in p53 protein level which was functionally associated with the activation of downstream p53-responsive genes such as p21WAF1/CIP1, gadd45 and bax. Thus, this motif mediates the binding of DSS1 to proteasome complex and, at least in part, modulates Gankyrin-HDM2-mediated p53 protein ubiquitination and degradation by recruiting the 19SRP/20S core particle (CP) via the Rpn3/S3 molecule. Further exploitation of the potent anti-proliferative activity by blocking DSS1-RPN3/S3 interaction to reactivate the function of p53 and its regulated genes could aid in the development of an anti-cancer drug with important clinical potential and receive increased attention in the near future.

22 Satellite Cells and Mature Myofibers Give Rise to Different Forms of Rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in childhood. The cell of origin in alveolar rhabdomyosarcoma (ARMS), the most aggressive form of RMS, is controversial and the proposed origins range from muscle stem cells (satellite cells) and mesenchymal stem cells to

maturing myofibers. We previously established a conditional mouse model of ARMS by activating Pax3:Fkhr oncogene and inactivating p53 in Myf6-expressing maturing myofibers. These mice developed ARMS at 100% penetrance with a mean latency of 114 days. However, when these mutations were activated in Pax3 or Pax7-expressing early myogenic lineages, the mice showed developmental abnormalities but had no tumors. To further explore the possibility that ARMS may be arising from earlier myogenic lineages after birth, we have activated conditional Pax3:Fkhr knock-in and conditional p53 deletion alleles in early prenatal and postnatal myogenic progenitors. Pax3-hypaxial domain expression gave rise to ARMS, but the tumor frequency was significantly lower than Myf6 maturing myofiber domain expression. The embryonic-fetal Myf5 domain also gave rise to ARMS, but most of the mice showed developmental abnormalities due to inadvertent germline activation of Pax3:Fkhr in those mice (Myf5 is expressed in reproductive organs). Using a tamoxifen-inducible Pax7-CreER mouse line, postnatal Pax7 expressing satellite cells also developed tumors, but the tumor frequency was lower than from Myf6 expressing maturing myofibers. Tumors from satellite cells occurred mainly in the face and genitourinary regions which are the frequent sites in human embryonal rhabdomyosarcoma (ERMS), whereas ARMS from the Pax3-hypaxial domain and Myf6 domain occurred most frequently in the extremities. Histologically, tumors from satellite cells were composed mainly of spindle cells with cross-striations and the histological diagnosis was pleomorphic or embryonal RMS, not alveolar RMS. Furthermore, the expression level of Pax3:Fkhr in the satellite cell tumors was significantly lower than the tumors from maturing myofibers. Microarray analysis of those tumors also indicated that the tumors derived from satellite cells harbor molecular characteristics similar to a Patched1-p53 model of ERMS. These results suggest that the cell of origin in ARMS is likely to be maturing myofibers rather than muscle satellite cells.

23 Characterization of stem-like cancer cells in pediatric tumor cell lines.

Patricia C. Sanchez-Diaz, Gail E. Tomlinson, and Jaclyn Y. Hung

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Introduction

The cancer stem cell (CSC) hypothesis postulates that tumors contain a subset of self-sustaining cells capable to self-renew and perpetuate the tumor. These cells tend to be resistant to the conventional therapy and, therefore, may be responsible for tumor relapse. In order to develop specific therapies to

target these CSC, a deeper understanding on the pathways regulating their survival is needed. Notch signaling is a developmental pathway that regulates stem cell self-renewal. Dysregulation in Notch pathway is frequent in childhood tumors and the isolation of cancer cells with stem-like properties has been reported in numerous cases. In this study, we present the isolation and characterization of a fraction of cells with high aldehyde dehydrogenase activity (i.e. ALDHBR) from two pediatric liver tumor cell lines. The potential role of Notch in this population was also investigated.

Experimental Procedures

Characterization of stem-like cancer cells was performed based on high and low aldehyde dehydrogenase activity (ALDHBR and ALDHDIM respectively) populations where purified from HepG2 and Hep293TT cell lines using the ALDEFLUOR kit and fluorescence-activated cell sorting (FACS). Co-expression of the surface markers CD133, Ep-CAM, Thy-1 and CD49f was analyzed in these two fractions by flow cytometry. qRT-PCR and mass spectrometry was performed on these cell fractions. In addition, the gamma secretase inhibitor compound E was used to block the activity of the Notch pathway. Four inhibitor concentrations were tested: 1, 2, 5 and 10 μ M and control cells were treated with vehicle (0.04 % DMSO) and the percentage of ALDEFLUOR positive cells measured as indicated above.

Results

Differential expression of a subset of "stemness" related genes was found between the ALDHBR and ALDHDIM populations. On average a 2-fold reduction in the ALDEFLUOR positive fraction (CSC surrogate assay) was observed in cells treated with the Notch inhibitor in both cell lines.

Conclusions

Tumorigenicity studies are ongoing. Our data suggest a link between Notch and stem-like cancer cells in childhood liver tumors. These finding may be consistent with a role for Notch sustaining CSCs and, therefore, may have clinical relevance.

mor has been shown to sustain the relentless growth of the mass, and more importantly, the capacity of disseminating and subsequently metastasizing. As well, there is some data suggesting that the stem-like cells are relatively resistant to chemo and radiation therapy. Studies suggests that the molecules that determine the self-renewal nature of normal stem cells and the pathways involved in regulating this process are dysfunctional in the cancer stem cells. microRNAs (miRNAs) are an abundant class of endogenous non-coding, short single-stranded RNAs that function as negative gene regulators. miRNAs are aberrantly expressed in certain cancers and have also been shown to regulate a number of cancer-related genes involved in the control of cell cycle, apoptosis, angiogenesis, metastasis and chemotherapeutic drug response. There are increasing data suggesting a causal role for microRNAs in childhood malignancies. The ability of miRNAs to simultaneously posttranscriptional regulates many targets genes make them particularly attractive candidates as regulators of self-renewal. If so, then they might provide novel therapeutics to target the cancer stem cells. This study was undertaken to identify the miRNAs differentially expressed in the stem-like cancer cell enriched fractions from Daoy (medulloblastoma) and Hep293TT (hepatoblastoma) cell lines.

Methods

Hep293TT stem-like fractions were enriched using FACS as cells with high enzymatic activity of aldehyde dehydrogenase (ALDH). Neurospheres (enriched for stem-like cells) were generated from Daoy cells by culture in medium supplemented with basic fibroblast growth factors and epidermal growth factors. miRNA profiling was performed using the stem-loop qRT-PCR assay in a high-throughput 384 wells format. A total of 768 miRNAs found in the human genome, annotated in the miRBase Registry (<http://microrna.sanger.ac.uk>) were analyzed. The data from the stem-like enriched fractions and their non stem-like counterparts was analysed using the comparative CT method of relative quantification.

Results

We identified 48 miRNAs for which the expression was elevated at least two folds in the stem-like cells of both cell lines. Of the miRNAs that were down regulated, the overlap between these two lists yield as set of 46. Preliminary analyses suggested that some of the potential targets are gene related to Hedgehog, Wnt and Notch pathways, cell cycle, and apoptosis.

Conclusion

Future studies will determine functional relevance of each of the identified miRNAs. Further data mining is needed to predict the possible gene targets and pathways to unravel potential mechanisms.

24 MicroRNAs and stem-like cells in pediatric cancer cell lines.

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Purpose

Emerging evidence suggests that most cancers, if not all have their own "stem cells". This stem-like fraction within a tu-

25 miRNAs involved in kidney development are dysregulated in Wilms' tumor

J. Saadi Imam, Kihoon Yoon, Jennifer S. Lee, Caroline Camosy, Yidong Chen & Manjeet Rao

Micro RNAs (miRNAs) are naturally occurring, short non-coding RNAs believed to be involved in many biological processes including normal development and adult cancer. It seems logical that miRNAs would be particularly important in pediatric cancers as these malignancies involve perturbations of developmental processes, yet relatively little is known about the role of miRNAs in pediatric cancers. We have recently obtained exciting data suggesting that oncogenesis in Wilms' tumor is mediated by sequences with regulatory functions such as miRNAs. Specifically, we have identified a cohort of miRNAs that show altered expression in Wilms' tumor patients when compared with normal matched control kidney. Interestingly, our results reveal that specific miRNAs, which are highly expressed in the normal kidney, have drastically reduced expression in Wilms' tumors. We further show that these miRNAs target the Six homeobox gene family, which is highly expressed in fetal kidney and Wilms' tumors and reported to play crucial roles in normal embryonal kidney development and tumor proliferation. High levels of Six family gene expression in many cancers including rhabdomyosarcoma, has also been shown to cause resistance to apoptosis as well as genomic instability and poor survival. We propose that dysregulation of oncogenic protein Six1 due to altered expression of miRNAs results in Wilms' tumor growth and metastasis. We envision that these studies will reveal novel regulatory mechanisms that function normally in kidney development and abnormally in Wilms' tumor. Since Wilms' tumor is derived from genetic lesions that block differentiation, identification of miRNAs that target genes involved in regulation of cellular differentiation may ultimately be amenable to therapeutic intervention. In addition, the distinct patterns of miRNA expression in different Wilms' tumor stages indicate that miRNAs may be used as molecular markers to better classify Wilms' tumor patients into low and high risk subgroups. We believe that our findings will help achieve the goals of National Wilms' Tumor study group 5 (NWTS5), which is to identify tumor-specific markers for better prognosis, investigate novel treatment approaches and ultimately improving recovery rates and quality of life for children suffering from Wilms' tumor.

26 The NIRF Ubiquitin Ligase Interacts With the Cell-Cycle Machinery and Induces Cell Cycle Arrest

Tsutomu Mori, MD, PhD

Cyclins are subject to regulated protein destruction via the ubiquitin-proteasome system, which is critical for the ordered cell cycle progression. Previously, we reported NIRF (also known as UHRF2) as an ubiquitin ligase that interacts with the CDK2-cyclin E complex. In the present study, we characterize NIRF for its potential role in the regulation of cell cycle progression. We show that NIRF interacts with the CDK-cyclin complex by binding to the cyclin subunit. NIRF forms complexes with cyclin D1 and E1, which is mediated by the YDG/SRA domain. Biochemical analysis reveals that NIRF preferentially targets these G1 cyclins for ubiquitination, implicating a role for this E3 ligase in regulation of the G1 phase. Indeed, over-expression of NIRF caused G1 arrest. Conversely, RNAi-mediated knockdown of NIRF expression resulted in the accelerated G1/S progression. Together, our results demonstrate NIRF is a novel E3 ligase, distinct from the SCF-type ubiquitin ligase complexes, important for regulation of G1/S transition.

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The Greehey Children's Cancer Research Institute would also like to express our thanks for generous support from Roche Diagnostics Corporation and The Takeda Oncology Company. As contributors these firms have enabled the science of children's cancer research to more fully advance through assisting as we provide an opportunity for researchers to interact and share ideas, techniques, and results.



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