An AACR Special Conference

Noncoding RNAs and Cancer

January 8-11, 2012 • Eden Roc Renaissance Beach Resort and Spa • Miami Beach, FL

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Four presenters of meritorious abstracts have been selected by the Conference Co-Chairpersons to receive awards to attend this conference. All graduate and medical students, postdoctoral fellows, and physicians-in-training were eligible for consideration. The names of the Scholar-in-Training awardees, their affiliations, and their poster or proffered presentation numbers are provided below. The AACR sincerely thanks Aflac, Inc., and AstraZeneca International for their support of these awards.

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David J. Drasin, University of Colorado, Anschutz Medical Campus, Aurora, CO, B1

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Catherine S.S. Enfield, BC Cancer Research Centre, Vancouver, BC, Canada, B21

AACR Minority Scholar in Cancer Research Award

The AACR is pleased to administer this important award program that is supported by a generous grant from the Center to Reduce Cancer Health Disparities (CRCHD) of the National Cancer Institute and provides funds for the participation of meritorious minority scientists in AACR conferences. These awards are available to predoctoral (graduate or medical) students or residents, clinical or postdoctoral fellows, and junior faculty who are making contributions to cancer research and are from minority groups considered to be underrepresented in cancer and biomedical research by the National Cancer Institute. The AACR Minority Scholar in Cancer Research awardee for this conference is:

Belinda R. Hauser, Howard University, Washington, DC, B30

AACR Minority-Serving Institution Faculty Scholar in Cancer Research Award

The AACR MSI Faculty Scholar Awards program is supported by a generous grant from the Center to Reduce Cancer Health Disparities of the National Cancer Institute. The purposes of the award are to increase the scientific knowledge base of faculty members at MSIs, to encourage them in their research, and to assist in inspiring their students to pursue careers in cancer research. The AACR offers these awards for participation in its meetings and conferences to full-time faculty of minority-serving institutions (historically black colleges and universities [HBCUs], Hispanic-serving institutions [HSIs], Indian tribally controlled colleges and universities [ITCCUs], and other postsecondary institutions as defined by the U.S. Department of Education) who are scientists at the assistant professor level or above. Only citizens of the United States or Canada or scientists who are permanent residents in these countries may receive one of these awards. The AACR Minority-Serving Institution Faculty Scholar in Cancer Research awardees for this conference are:

Maria del Mar Gonzalez-Pons, University of Puerto Rico Comprehensive Cancer Center, San Juan, PR

Xinbin Gu, Howard University, Washington, DC, A13

American Association for Cancer Research

UPCOMING CONFERENCES

Advances in Prostate Cancer Research
Chairpersons: Arul M. Chinnaiyan and Charles L. Sawyers
February 6-9, 2012
Orlando, FL

AACR 103rd Annual Meeting 2012
Chairperson: Benjamin G. Neel
March 31-April 4, 2012
Chicago, IL

Molecularly Targeted Therapies: Mechanisms of Resistance
Co-Chairpersons: Pasi A. Jänne, Mace L. Rothenberg, and Carlos L. Arteaga
May 9-12, 2012
San Diego, CA

Pancreatic Cancer: Progress and Challenges
Co-Chairpersons: Daniel D. Von Hoff, David A. Tuveson, Dafna Bar-Sagi, and Chi Van Dang
June 18-21, 2012
Lake Tahoe, NV

Chemical Systems Biology: Assembling and Interrogating Computational Models of the Cancer Cell by Chemical Perturbations
Chairperson: Andrea Califano
Additional chairpersons and location to be announced
June/July 2012

Eleventh Annual International Conference on Frontiers in Cancer Prevention Research
Chairperson to be announced
October 16-19, 2012
Anaheim, CA

Fifth AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved
Chairperson: William G. Nelson
October 27-30, 2012
San Diego, CA

AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics
Scientific Committee Chairpersons: Stefan Sleijfer, James H. Doroshow, and Kenneth C. Anderson
November 6-9, 2012
Dublin, Ireland

Post-GWAS Horizons in Molecular Epidemiology: Digging Deeper into the Environment
Co-Chairpersons: Shelley S. Tworoger and Cornelia M. Ulrich
November 11-14, 2012
Hollywood, FL

AACR-NCI Conference on Cancer Genomics: The Cancer Genome Atlas and Beyond
Co-Chairpersons: Matthew L. Meyerson, Arul M. Chinnaiyan, and Barbara Wold
November 2012

Tumor Immunology
Co-Chairpersons: Elizabeth M. Jaffee, Glenn Dranoff, and Stanley Riddell
December 2-5, 2012
Miami, FL

AACR-SNM Joint Conference on State-of-the-Art Molecular Imaging in Cancer Biology
Co-Chairpersons: David Piwnica-Worms and Carolyn Anderson
Early 2013

Tumor Invasion and Metastasis
Co-Chairpersons: Bruce R. Zetter and Zena Werb
January 20-23, 2013
San Diego, CA

Ninth AACR-JCA Joint Conference
Co-Chairpersons: Tyler Jacks and Kohei Miyazono
February 21-25, 2013
Maui, HI

Synthetic Lethality and Cancer
Chairperson: William C. Hahn
Additional chairpersons and location to be announced
May 2013

Animal Models of Cancer
Co-Chairpersons: Thomas Look, Cory Abate-Shen, and Terry A. Van Dyke
Fall 2013

Bioinformatics
Co-Chairpersons: Andrea Califano, Arul M. Chinnaiyan, and Franziska Michor
Fall 2013

AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics
Chairpersons to be announced
October 19-23, 2013
Boston, MA

Also in development:
Pediatric Cancer
Melanoma
KRAS
Developmental Pathways in Cancer
Chromatin Remodeling in Cancer
Targeted Therapies in Hematological Malignancies

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Conference Program

Sunday, January 8

4:00 p.m.-8:00 p.m. **Registration**

8:00 p.m.-9:00 p.m. **Keynote Presentation**

Physiological and pathological functions of microRNAs
David Baltimore, California Institute of Technology, Pasadena, CA

9:00 p.m.-10:30 p.m. **Opening Reception**

Monday, January 9

7:00 a.m.-8:00 a.m. **Continental Breakfast**

8:00 a.m.-10:00 a.m. **Session 1: Long Noncoding RNAs**
Session Chairperson: Jeannie T. Lee, Massachusetts General Hospital, Boston, MA

Navigating the balance of INK4/ARF expression in cancer: ncRNAs as sensors and regulators of transcription
Martin J. Walsh, Mount Sinai School of Medicine, New York, NY

Regulatory networks in onco-lncRNAomics: cis-regulation and nonconservation
Leonard Lipovich, Wayne State University School of Medicine, Detroit, MI

Linking RNA to human health and disease
John L. Rinn, Beth Israel Deaconess Medical Center, Boston, MA

$BRAF^{V600E}$ remodels the melanocyte transcriptome and induces BLNCR1 to regulate melanoma cell migration*
Ross J. Flockhart, Stanford University, Stanford, CA

10:00 a.m.-10:15 a.m. **Break**

*Short talks from proffered papers

10:15 a.m.-12:15 p.m. **Session 2: Chromatin Modifications**
Session Chairperson: Danesh Moazed, Harvard Medical School, Boston, MA

Mechanism of RNAi-mediated heterochromatin assembly
Danesh Moazed

Epigenetic genome control by heterochromatin machinery and noncoding RNAs
Shiv Grewal, National Cancer Institute, Bethesda, MD

Elucidation of enhancer-like RNAs
Ramin Shiekhattar, The Wistar Institute, Philadelphia, PA

12:15 p.m.-2:30 p.m. **Lunch On Own/Free Time**

2:30 p.m.-4:30 p.m. **Session 3: miRNA Biology**
Session Chairperson: David P. Bartel, Massachusetts Institute of Technology, Cambridge, MA

microRNAs and their regulatory targets
David P. Bartel

Unraveling the complexities of p63 in cancer and stem cells
Elsa R. Flores, The University of Texas MD Anderson Cancer Center, Houston, TX

microRNAs and control of metabolism
Markus Stoffel, Institute of Molecular Systems Biology, Zurich, Switzerland

4:30 p.m.-6:30 p.m. **Poster Session A/Reception**

6:30 p.m.- **Dinner On Own/Evening Off**

Tuesday, January 10

7:00 a.m.-8:00 a.m. **Continental Breakfast**

8:00 a.m.-10:00 a.m. **Session 4: Biology of Long Noncoding RNAs**
Session Chairperson: Jeannie T. Lee, Massachusetts General Hospital, Boston, MA

Spreading of X-chromosome inactivation via a hierarchy of defined Polycomb stations
Jeannie T. Lee

Control of gene expression in the nucleus by small RNAs
David R. Corey, UT Southwestern Medical Center, Dallas, TX

Small RNA pathways in germline
Alexei A. Aravin, California Institute of Technology, Pasadena, CA

Functional characterization of p53-regulated long intervening noncoding RNAs (lincRNAs)*
Nadya M. Dimitrova, David H. Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA

10:00 a.m.-10:15 a.m. **Break**

10:15 a.m.-12:15 p.m. **Session 5: miRNA Regulation in Development**
Session Chairperson: Eric N. Olson, UT Southwestern Medical Center, Dallas, TX

microRNA control of muscle development and disease: From new biology to new therapeutics
Eric N. Olson

Long-term, efficient inhibition of miRNA function in mice using Tough Decoys delivered by adeno-associated virus vectors
Philip D. Zamore, University of Massachusetts Medical School, Worcester, MA

microRNA regulation in stem cells and cancer
Richard I. Gregory, Children's Hospital Boston/Harvard Medical School, Boston, MA

12:15 p.m.-2:30 p.m. **Poster Session B/Lunch**

*Short talks from proffered papers

2:30 p.m.-4:30 p.m. **Session 6: miRNA Regulation in Cancer**
Session Chairperson: Carlo M. Croce, The Ohio State University Comprehensive Cancer Center, Columbus, OH

microRNAs as therapeutic targets
Carlo M. Croce

microRNA reprogramming in cancer: Mechanisms and consequences
Joshua Mendell, UT Southwestern Medical Center, Dallas, TX

The ceRNA hypothesis and the noncoding revolution in cancer research and therapy
Pier Paolo Pandolfi, Beth Israel Deaconess Medical Center, Boston, MA

Characterization of a novel pseudogene expressed antisense RNA that regulates PTEN expression*
Per Johnsson, Karolinska Institutet, Stockholm, Sweden

4:30 p.m.- **Dinner On Own/Evening Off**

Wednesday, January 11

7:00 a.m.-8:00 a.m. **Continental Breakfast**

8:00 a.m.-10:00 a.m. **Session 7: RNA in Cancer and Disease**
Session Chairperson: William C. Hahn, Dana-Farber Cancer Institute, Boston, MA

Functional genomics, small RNAs, and cancer
William C. Hahn

Mechanisms of miRNA-mediated cellular reprogramming
Edward E. Morrisey, University of Pennsylvania, Philadelphia, PA

New therapeutic strategies for lymphomas inspired by functional and structural genomics
Louis M. Staudt, National Cancer Institute, Center for Cancer Research, Bethesda, MD

Molecular function of the RNA binding protein EWS in RNA processing*
Bethsaida I. Nieves, Stanford University School of Medicine, Stanford, CA

10:00 a.m.-10:15 a.m. **Break**

*Short talks from proffered papers

10:15 a.m.-12:15 p.m. **Session 8: RNA Therapeutics**

Session Chairperson: William C. Hahn, Dana-Farber Cancer Institute, Boston, MA

Targeting of microRNAs for therapeutics
Sakari Kauppinen, Santaris Pharma, Horsholm, Denmark

Long noncoding RNAs as drug targets
Claes R. Wahlestedt, University of Miami Miller School of Medicine, Miami, FL

Anti-miR therapeutics for cancer
Eric Marcusson, Regulus Therapeutics, San Diego, CA

Treatment of ovarian cancer with targeted tumor-penetrating siRNA nanocomplexes*
Yin Ren, David H. Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA

12:15 p.m. **Closing Remarks/Departure**

*Short talks from proffered papers

Invited Abstracts

Keynote Presentation

Physiological and pathological functions of microRNAs. David Baltimore. California Institute of Technology, Pasadena, CA.

microRNAs have emerged as major regulators of the functions of genes in multicellular organisms. They generally and perhaps always act as reducers of the protein output from a given mRNA allotment generated from a particular gene. However, when acting on mRNAs that function in a regulated pathway, they can either inhibit or augment the output from that pathway depending on whether they act on inhibitory gene transcripts or activating gene transcripts. We have concentrated on miRs that function within the circuitry of the hematopoietic system, focusing on those that impinge on innate and adaptive immune responses. We have found that miR-146a acts as a classic negative regulator of inflammation while miR-155 acts as a positive regulator by targeting inhibitory proteins. A miR that has particularly interested us recently is miR125b, which both acts during the development of hematopoietic cells from the hematopoietic stem cell and modulates the function of myeloid cells. In all of this work, we have relied on mouse knockouts, siRNA knockouts and overproducing constructs to probe miR function and have been impressed by the ease with which these genetic manipulations can lead to hyperproliferation and cancer. Overexpression of miR-125b leads to particularly rapid induction of very aggressive tumor cells that can be of either the myeloid or lymphoid lineage. The developmental origin of these tumors is under investigation.

microRNAs have been seen as modulators of gene expression. Because it is becoming clear that they are so powerful in their physiological and pathological properties, it seems more appropriate to now consider them as co-regulators of gene expression, partners with transcription factors in determining the functional outcome of developmental and regulatory pathways.

Session 1: Long Noncoding RNAs

Navigating the balance of INK4/ARF expression in cancer: ncRNAs as sensors and regulators of transcription. Martin J. Walsh[1], Francesca Aguilo[1], SiDe Li[1], Kyoko Yap[1], Jesus Gil[2], Ming-Ming Zhou[1]. [1]Mount Sinai School of Medicine, New York, NY, [2]Clinical Sciences Centre, Hammersmith Hospital Campus, Imperial College Faculty of Medicine, London, United Kingdom.

The *INK/ARF (CDKN2A)* locus expresses $p15^{Ink4B}$, $p16^{Ink4A}$, $p14^{Arf}$ and is under control of *Polycomb* Repressive Complexes PRC1 and PRC2 to impose transcriptional silencing to bypass cellular senescence upon oncogenic stress. Previously, we had identified a constituent member of human PRC1, known as the chromobox protein 7 (CBX7), is endowed the capacity to bind specifically to elements of the long non-coding RNA identified as the non-coding **an**ti-sense **R**NA of the **I**NK **l**ocus (ANRIL) RNA transcript as a mature processed polyadenylated RNA transcript. ANRIL represent a large heterogeneous population of individual transcripts and their isoforms arising from the localized genomic region overlapping INK/ARF. These transcripts reveal independent patterns of expression dependent on the state of cell growth and differentiation. Moreover, specific transcripts overlapping the ANRIL region of chromosome 9p21.3 provide strict correlation to human embryonic stem cell pluripotency and self-renewal versus differentiation. We recently identify specific ANRIL transcripts and isoforms that are correlative with human prostate tumor stages and states of malignancy. We had previously shown that certain transcripts correlate with PRC1 is organized on the *INK/ARF* locus through the interaction between CBX7 and the nascent *ANRIL* transcript to help establish and maintain repressive histone H3K27 methylation and H2AK119 monoubiquitination. Both PRC1 and PRC2 can interact with different *ANRIL* transcript regions and it remains unclear whether this activity is cooperative. This activity is abrogated in the absence of RNA polymerase II activity thereby

requiring nascent transcription of anti-sense transcripts of *INK/ARF* by RNA polymerase II (RNA POLII). Interestingly, TAF15/TAFII68 can be tethered to CBX7 in prostate carcinoma cells where the *ANRIL* ncRNA transcripts are elevated. Our recent finding is that specific *ANRIL* transcripts directed by RNAPOLII are influenced by RNA polymerase III (RNA POLIII) as measured through the depletion of the core RNA POLIII factor BRF1. This evidence suggests that RNA POLIII may populate RNA synthesis of shorter transcripts to influence PRC1 and PRC2 binding thereby instructing the behavior of RNA POLII synthesis. This provides a rational mechanism whereby RNA POLIII with RNAPOLIII provide the genomic RNA context to instruct PRC1 and PRC2 to exert antisense transcription at the expense of transcriptional activity of *INK/ARF*.

Regulatory networks in onco-lncRNAomics: cis-regulation and nonconservation.

Leonard Lipovich. Wayne State University, Detroit, MI.

Global studies of the transcriptome reveal that approximately half of human transcriptional units (genes) encode solely non-protein-coding RNAs (ncRNAs), which, in addition to microRNAs and other small RNAs, include the far more numerous long non-coding RNAs (lncRNAs). While the lncRNAome (the total set of all lncRNAs) is increasingly recognized as a rich repository of novel regulators, functional information is still lacking for the majority of lncRNAs (Lipovich et al 2010). We used transcript-to-genome alignments to identify and manually curate 4,511 human cis-antisense gene pairs, each comprised of two distinct genes mapping to opposite strands of the same locus with overlapping exons. These pairs contained 3,769 lncRNA genes, most of which (2,818) resided in coding-noncoding (mRNA-lncRNA) pairs, suggesting that antisense lncRNAs may be cis-regulators of protein-coding genes. 803 lncRNAs from cis-antisense loci contained, in addition to nonrepetitive exons, exonic primate-specific Alu repeats, indicating that lncRNAs at cis-antisense loci have undergone gene structure modification specifically in primate evolution. We mapped 410 SNPs to exon-exon, cis-antisense overlaps. Through their simultaneous localization in exons of two overlapping genes, these SNPs may contribute to multigenic or contiguous-gene phenotypes.

1,213 cis-antisense pairs had both sense and antisense transcripts represented by distinct reliable probesets on Affymetrix U133 microarrays. To search for lncRNA regulators of breast cancer gene expression, we first interrogated lncRNA-mRNA co-expression at these loci in a human Affymetrix breast cancer dataset (GSE4922). Co-expression of cis-encoded lncRNAs and mRNAs was observed in half of the cis-antisense pairs. Simultaneous detection of sense and cis-antisense RNAs for each antisense pair in cancer samples opens new avenues for expression-based diagnostic marker development and for functional investigations of cis-antisense regulation involving non-conserved lncRNAs. Four co-expressed lncRNA-mRNA cis-antisense pairs contained lncRNAs which had positional equivalents (Engström et al 2006), but not conserved orthologs, in the mouse genome. BC042008, one such non-conserved lncRNA, was co-expressed in breast cancer with its partner ZEB1, a known repressor of IL-2 and plakophylin, the latter in cancer progression. This implies biomarker and even functional potential for human cis-antisense lncRNAs that are not conserved in mouse.

The abundance of lncRNAs in breast cancer cis-antisense data prompted us to interrogate the complete human lncRNAome, including though not limited to cis-antisense pairs, for breast cancer function. Recently, we generated a catalog of human lncRNAs (Jia et al 2010). By quantitative RTPCR, we identified estrogen-activated (DLEU1, Deleted in Leukemia 1) and estrogen-repressed (BC038211) lncRNAs that are endogenously expressed in human MCF7 cells. Among Affymetrix probesets for 786 genes significantly ($p<0.01$) associated with estrogen receptor (ER) status of clinical breast tumor samples (Ivshina et al 2006), we observed 10 lncRNAs, including XIST. To directly identify ERα-targeted lncRNAs, we intersected genomewide ChIP-PET ERα TFBS localization (Lin et al 2007) with

the MCF7 transcriptome. Results included a novel lncRNA initiating at the TBX2 bidirectional promoter in the BCAS3 (breast carcinoma amplified sequence 3) locus. This non-conserved lncRNA with a nearby ERα binding site has a positional equivalent but not a conserved ortholog in mouse. Having previously used our lncRNA catalog to develop and implement custom human lncRNA expression analysis microarrays in multiple cancer cell lines, post-mortem brain, and surgically resected brain samples, we are currently deploying these arrays to find additional estrogen-responsive lncRNAs in the MCF7 transcriptome.

To understand the regulatory network placement of lncRNAs, including those that lack conservation and reside at cis-antisense gene pairs, we have addressed, in systems beyond breast cancer, two fundamental questions: Which transcription factors directly regulate lncRNAs by binding directly at promoters or intragenic regions of lncRNA genes? And what are the downstream effects of altering the cellular levels of these lncRNAs? To address the first question, we annotated genomewide TFBS datasets for several transcription factors, including mouse Oct4 and Nanog (key regulators of pluripotency in embryonic stem cells) and human REST. By manual identification and protein-coding capacity assessment of the cDNA- or EST-supported transcriptional unit nearest to each TFBS, regardless of whether the unit corresponded to a known gene, we found that 10% of mouse Oct4 and Nanog proximal targets in ES cells are lncRNAs, while 23% of human REST binding sites proximally target lncRNAs. We validated specific lncRNA direct targets of these transcription factors: perturbation of the transcription factors altered the levels of the targeted lncRNAs (Johnson et al 2009).

To examine the second question, we perturbed the lncRNAs Miat, a direct target of Oct4, and AK141205, a direct target of Nanog, in mouse ES cells by RNAi and overexpression. These perturbations caused distinct changes in Oct4 and Nanog mRNA levels, lineage-specific gene expression, and pluripotency, highlighting the lncRNA Miat as a novel Oct4 co-activator in a positive feedback loop of the ES regulatory network (Sheik Mohamed et al 2010). We also used a human cancer cell line system, SH-SY5Y neuroblastoma cells, where we focused on the non-conserved, primate-specific lncRNA BDNFOS, which shares a bidirectional promoter with LIN7C and a cis-antisense overlap with BDNF. Previously, with microarrays and quantitative RTPCR (Lipovich and Loeb, ms in prep), we demonstrated reciprocal patterns of BDNF and BDNFOS transcription in highly active regions of human neocortex removed as a treatment for intractable seizures. RNAi-mediated knockdown of BDNFOS increased BDNF expression, suggesting that BDNFOS directly downregulates BDNF. Therefore, RNAi and overexpression of lncRNAs can help establish both cis- (e.g. BDNF) and trans-regulatory (e.g. Miat) lncRNA network functions.

Finally, to investigate whether the human onco-lncRNAome may function through translation of the lncRNAs, rather than purely at the RNA level, we performed proteogenomic integration of tandem mass spectrometry and RNAseq data from two ENCODE cell lines, GM12878 and the chronic myelogenous leukemia cell line K562 (ENCODE AWG). LncRNAs were ~13 fold less likely to produce detectable peptides than mRNAs expressed at similar levels, indicating that ~92% of Gencode v7 lncRNAs are untranslated in these cell lines. Intersecting 9,640 lncRNA loci with 79,333 peptides yielded 85 unique peptides matching only 69 Gencode lncRNAs, with the majority of these peptides derived from the few rare protein-coding transcripts that were mis-annotated as lncRNAs in Gencode and that were hence absent from our independent, non-Gencode lncRNA catalog. We conclude that ectopic translation and cryptic mRNAs are rare in the lncRNAome of these cells. Summarily, our results, including those from human clinical breast cancer datasets and diverse cancer cell line systems, indicate that numerous specific lncRNAs, including non-conserved and cis-antisense transcripts, exert cis- and trans-regulatory functions in regulatory networks.

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Engström P et al. PLoS Genet. 2006 Apr; 2(4):e47.
Ivshina AV et al. Cancer Res. 2006 Nov 1; 66(21):10292-10301.
Jia H et al. RNA. 2010 Aug; 16(8):1478-1487.
Johnson R et al. RNA. 2009 Jan; 15(1):85-96.
Lin CY et al. PLoS Genet. 2007 Jun; 3(6):e87.
Lipovich L, Johnson R, Lin CY. Biochim Biophys Acta. 2010 Oct; 1799:597-615.
Sheik Mohamed J et al. RNA. 2010 Feb; 16(2):324-337.

Notes: A full listing of collaborators, with funding acknowledgments, will be provided during the presentation. Due to time limitations, presentation of all results that are mentioned in this abstract is not guaranteed.

Linking RNA to human health and disease. Moran Cabili[1], Eric Lander[1], Pardis Sabeti[1], Aviv Regev[1], John L. Rinn[1], John L. Rinn[2], John L. Rinn[3], Cole Trapnell[1], Cole Trapnell[2], Loyal Goff[1], Loyal Goff[2], Kate Broadbent[1], Kate Broadbent[2], Mitchell Guttman[1]. [1]Broad Institute of MIT and Harvard, Cambridge, MA, [2]Harvard University, Cambridge, MA, [3]Harvard Medical School, Boston, MA.

In the 50 years since RNA was identified as a central component in the flow of genetic information, it has become increasingly clear that RNA is more than a mere messenger and instead performs vast and diverse functions. Numerous studies have revealed that the mammalian genome is pervasively transcribed, giving rise to many thousands of non-coding transcripts including a class of long intergenic noncoding RNAs (lincRNAs). Raising the question of what do lincRNAs do?

To address this question we have developed a 'guilt by association' to 'predict' lincRNA functions leading to hypothesis driven experimentation. Our guilt by association method pointed to a clear connection of lincRNAs and numerous cellular pathways ranging from pluripotency, cancer, adipogenesis to parasitology. Experimental perturbation experiments have unraveled a myriad of functional roles for lincRNAs in these pathways. Together, these results point to key regulatory roles for lincRNAs across diverse biological pathways and diseases, with a common theme of interfacing with and modulating protein regulatory complexes.

Session 2: Chromatin Modifications

Epigenetic genome control by heterochromatin machinery and noncoding RNAs. Shiv Grewal. National Cancer Institute, Bethesda, MD.

Heterochromatin assembly involving posttranslational modifications of histones is critical for various chromosomal processes including the regulation of gene expression and the maintenance of genomic integrity. Defective heterochromatin formation has been linked to cancer. Our previous work has shown that non-coding RNAs and the RNAi machinery, involved in the processing of non-coding RNAs, play prominent roles in the assembly of heterochromatin structures. Indeed, the loss of factors involved in RNAi such as Argonaute, Dicer and RNA-dependent RNA polymerase cause severe defects in centromeric heterochromatin formation, leading to missegregation of chromosomes during cell division. An Argonaute-containing RNAi effector complex named RITS has been identified that facilitates the loading of a conserved histone methyltransferase Clr4/Suv39h, which is essential for heterochromatin assembly. We have recently discovered an unexpected role for heterochromatin factors in the RNA quality control. Heterochromatin factors localize broadly across the genome and collaborate with RNAi machinery to suppress potentially deleterious RNAs, the uncontrolled accumulation of which can cause DNA damage and modify epigenetic genomic profiles. I will present our recent findings showing that non-coding RNAs and heterochromatin play important roles in dynamic regulation of genomes, which has important implications for human health and disease.

References:
Grewal, S.I.S. and Jia, S. (2007). Heterochromatin revisited. Nat. Rev. Genet., 8:35-46.
Chen, E.S., Zhang, K., Nicolas, E., Cam, H., Zofall, M., and Grewal, S.I.S. (2008)

Cell cycle control of centromeric repeat transcription and heterochromatin assembly. Nature, 451: 734-747.
Zofall, M., Fischer, T., Zhang, K., Zhou, M., Cui, B., Veenstra, T., and Grewal, S.I.S. (2009) Histone H2A.Z cooperates with RNAi and heterochromatin factors to suppress antisense RNAs. Nature, 461: 419-422.
Zhang, K., Fischer, T., Porter, R., Dhakshnamoorthy, J., Zofall, M., Zhou, M., Veenstra, T., Grewal, S.I.S. (2011). Clr4/Suv39 and RNA quality control factors cooperate to trigger RNAi and suppress antisense RNA. Science 331: 1624-1627.

Elucidation of enhancer-like RNAs. Ramin Shiekhattar. The Wistar Institute, Philadelphia, PA.

Spatial and temporal regulation of gene expression is achieved through the instruction provided by the distal transcriptional regulatory elements known as enhancers. How enhancers transmit such information to their targets has been a subject of intense investigation. Recent advances in high throughput analysis of the mammalian transcriptome have revealed a surprising result indicating that a large number of enhancers are transcribed to non-coding RNAs. While long non-coding RNAs were initially shown to confer epigenetic transcriptional repression, recent studies have uncovered a role for a class of such transcripts in gene-specific activation often from distal genomic regions. We will discuss the recent findings on the role of long non-coding RNAs in transcriptional regulation with an emphasis on new developments on the functional links between long non-coding RNAs and enhancers.

Session 3: miRNA Biology

Unraveling the complexities of p63 in cancer and stem cells. Elsa R. Flores. The University of Texas MD Anderson Cancer Center, Houston, TX.

Aberrant expression of microRNAs (miRNAs) and the enzymes that control their processing have been reported in multiple biological processes including in cancer and the regulation of stem cells, but the mechanisms governing this are not clearly understood. We have generated conditional knockout mice that allow the functional separation of *TAp63* and *ΔNp63*. Using the *TAp63* conditional knock out mouse model, we found that TAp63 suppresses tumorigenesis and metastasis through coordinate transcriptional regulation of *Dicer* and *miR-130b*. Interestingly, we have recently found that ΔNp63 also plays an important role in miRNA processing by transcriptionally activating DGCR8. In turn, DGCR8 is required for processing of key miRNAs that repress genes required for stem cell pluripotency, *Oct4*, *Sox2*, and *Nanog*. Strikingly, epidermal cells derived from *ΔNp63* deficient mice express markers of pluripotency at levels comparable to mouse embryonic stem cells. Moreover, these cells exhibit characteristics of stem cells that can self-renew and can differentiate into multiple cell fates in vitro and in vivo. Our data reveal a novel role for ΔNp63 in the commitment to epidermal differentiation through transcriptional regulation of DGCR8 and downstream modulation of key miRNAs required to maintain stem cell pluripotency. Taken together, our data show that p63 plays critical roles in regulating miRNAs in multiple biological processes.

microRNAs and control of metabolism. Markus Stoffel. Institute for Molecular Systems Biology, Zurich, Switzerland.

Obesity has been shown to be a strong risk factor for adult-onset diabetes. Importantly and less widely appreciated, there is also a significant link between obesity and cancer. Recent studies have clearly demonstrated that obesity is associated with the incidence and mortality of a number of malignancies, including colon, pancreatic, kidney, as well as aggressive prostate cancer in men and breast and endometrial cancer in women. Other studies have also found that individuals with various abnormalities associated with the insulin resistance such as hypertriglyceridemia, hyperglycemia and high insulin levels are at increased risk of certain cancers. Moreover, recent reports have suggested the possibility of an association between use of synthetic insulin and cancer. Together, a body of evidence suggests that

a link between obesity and cancer that may result from similar etiological mechanisms common to cancer, diabetes and insulin use.

We are studying the role of microRNAs in the control of glucose and lipid metabolism. Initial studies conducted in our lab have revealed a role of miR-375, the most abundant microRNA in the pancreatic beta cell, in maintaining normal beta cell mass. We recently broadened our investigations on the function of other microRNAs that regulate pancreatic b-cell homeostasis and insulin sensitivity. Two miRNAs and their role in regulating glucose homeostasis will be discussed:

1. miR141/200c cluster: Expression analysis of the miR-200 family cluster revealed a predominant localization in endocrine organs, such as the pancreatic beta cells, the pituitary and the gastrointestinal tract. Specific overexpression of these microRNAs in the pancreatic beta cell results in profound hyperglycemia, ketoacidosis and insulinopenia due to massive b-cell apoptosis. Conversely, mice lacking miR141/200c are protected from streptozotocin-induced b-cell death. These results implicate this microRNA cluster as a key regulator of programmed cell death in vivo.

2. miR-103/107: These two related miRNAs are upregulated in livers of obese mice and humans. We showed that silencing of miR-103/107 leads to improved glucose homeostasis and insulin sensitivity. In contrast, gain of miR-103/107 function in either liver or fat is sufficient to induce impaired glucose homeostasis. We identified caveolin-1, a critical regulator of the insulin receptor, as a direct target gene of miR-103/107 and demonstrated that caveolin-1 is upregulated upon miR-103/107 inactivation in adipocytes and that this is concomitant with stabilization of the insulin receptor, enhanced insulin signaling, decreased adipocyte size and enhanced insulin-stimulated glucose uptake. These findings demonstrate the central importance of miR-103/107 to insulin sensitivity and identify a new target for the treatment of type 2 diabetes and obesity.

Session 4: Biology of Long Noncoding RNAs

Spreading of X-chromosome inactivation via a hierarchy of defined Polycomb stations. Stefan F. Pinter[1], Stefan F. Pinter[2], Stefan F. Pinter[3], Yesu Jeon[1], Yesu Jeon[2], Yesu Jeon[3], Toshiro K. Ohsumi[2], Toshiro K. Ohsumi[3], Mark Borowsky[2], Mark Borowsky[3], Jeannie T. Lee[1], Jeannie T. Lee[2], Jeannie T. Lee[3], Ruslan I. Sadreyev[1], Ruslan I. Sadreyev[2], Ruslan I. Sadreyev[3], Eda Yildirim[1], Eda Yildirim[2], Eda Yildirim[3]. [1]Howard Hughes Medical Institute, Boston, MA, [2]Massachusetts General Hospital, Boston, MA, [3]Harvard Medical School, Boston, MA.

X-chromosome inactivation (XCI) achieves dosage balance in mammals by repressing one of two X-chromosomes in females. During XCI, the long noncoding Xist RNA and Polycomb proteins spread along the inactive X (Xi) to initiate chromosome-wide silencing. Although inactivation is known to commence at the X-inactivation center (Xic), how it propagates remains unknown. We have examined allele-specific binding of Polycomb repressive complex 2 (PRC2) and chromatin composition during XCI, and generate a chromosome-wide profile of Xi and Xa (active X) at nucleosome-resolution. Initially, Polycomb proteins are localized to a limited number of sites along the X. As Xist RNA spreads *in cis* during the XCI process, thousands of additional sites are recruited. PRC2 and H3K27 methylation spread along a gradient in an Xi-specific manner not seen on autosomes. Our findings suggest that XCI is governed by a hierarchy of defined Polycomb stations that spread H3K27 methylation *in cis*.

Control of gene expression in the nucleus by small RNAs. David Corey. UT Southwestern, Dallas, TX.

Double-stranded RNAs are widely used to control gene expression and are a promising class of therapeutic agent. Most studies have focused on recognition of mRNA in the cytoplasm and inhibition of translation. My presentation focuses on the action of small RNAs in the nucleus to modulate transcription or redirect alternative splicing.

In 2005, my laboratory observed that

synthetic RNAs complementary to the promoter region at the transcriptional start site of human progesterone receptor (PR) could block PR expression [1]. Inhibition was potent and robust, consistent with the suggestion that the synthetic RNAs were exploiting an endogenous mechanism for controlling gene expression. Promoter-targeted synthetic miRNAs were also capable of robust gene silencing [2], further supporting the existence of endogenous regulatory pathways. Modulation required complete seed sequence complementarity to the PR promoter, consistent with a mechanism that involves RNA-RNA recognition through Watson-Crick base-pairing.

In 2007, we observed that small RNAs could also target the PR gene promoter and activate PR expression [3]. Activation was observed in cells that had a low, but detectable, level of PR expression, while gene silencing had been observed in cells with a high level of PR expression. Inactive and active RNAs compete for recognition, demonstrating that small changes in target site location affect activity. These results are reminiscent of protein transcription factors, which bind to specific sequences, are sensitive to target site location or small conformation changes, and can activate gene expression in some contexts but not others.

Our promoter-targeted RNAs bind to long noncoding RNA transcripts at the PR promoter [4]. Use of antisense oligonucleotides to reduce transcript levels also reduces RNA-mediated modulation of gene expression. The small RNAs recruit AGO2 to the noncoding transcript, and inhibition of AGO2 expression reverses gene silencing or activation [5]. While AGO2 is normally expected to cleave fully complementary targets in the cytoplasm, our data suggests that AGO2 does not cleave transcripts associated with gene promoters in the nucleus.

A noncoding RNA transcript also overlaps the 3' terminus of the PR gene [6]. Small RNAs complementary to this noncoding transcript modulate PR expression, depending on the cell line used. The PR gene loops, juxtaposing its 3' and 5' termini and providing a direct path for a signal transmitting a signal from the 3' terminus of the gene to the promoter.

We have investigated whether RNAs can modulate other nuclear events. We designed small RNAs to be complementary to sequence within exons or introns near splice sites. These RNAs redirect alternative splicing and AGO2 was recruited to pre-mRNA [7]. These data provide more support for the action of small RNA in cell nuclei and for the conclusion that AGO2 may not always cause RNA cleavage when it encounters complementary targets in the nucleus.

Our data suggest that small RNAs can bind noncoding transcripts and modulate gene expression. Binding occurs in proximity to the gene promoter, in cis relative to the target gene. The RNA-AGO complex acts like a transcription factor to affect the transcription machinery and tip the balance of inducible genes like PR towards repression or activation.

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Small RNA pathways in germline. Alexei A. Aravin. California Institute of Technology, Pasadena, CA.

In animals, a discrete class of small RNAs, the piwi-interacting RNAs (piRNAs), guard germ cell genomes against the activity of mobile genetic elements.

piRNAs are generated, via an unknown mechanism, from apparently single-stranded precursors that arise from discrete genomic loci, termed piRNA clusters. Presently, little is known about the signals that distinguish a locus as a source of piRNAs. It is also unknown how individual piRNAs are selected from long precursor transcripts. To address these questions, we inserted new artificial sequence information into piRNA clusters and introduced these marked clusters as transgenes into heterologous genomic positions in mice and flies. Profiling of piRNA from transgenic animals demonstrated that artificial sequences were incorporated into the piRNA repertoire. Transgenic piRNA clusters are functional in non-native genomic contexts in both mice and flies, indicating that the signals that define piRNA generative loci must lie within the clusters themselves rather than being implicit in their genomic position. We found that both local and long-range sequence environments inform the generation of individual piRNAs from precursor transcripts.

Session 5: miRNA Regulation in Development

microRNA control of muscle development and disease: From new biology to new therapeutics. Eric N. Olson. UT Southwestern Medical Center, Dallas, TX.

We seek to define the gene regulatory networks that govern muscle and cardiovascular development and disease. The signaling pathways and transcriptional networks that control these processes are intertwined with a collection of microRNAs (miRNAs) that act as negative regulators of gene expression. We have identified miRNAs associated with muscular dystrophy and diverse cardiovascular disorders, including cardiac hypertrophy, heart failure, myocardial infarction, and angiogenesis. Gain- and loss-of-function studies in mice have revealed striking functions for these miRNAs in numerous processes, such as the control of muscle regeneration, nerve-muscle interactions, myosin expression, fibrosis, hypoxia, myocyte survival, and metabolism. Identification of miRNA targets has revealed new mechanisms and regulators of development and disease. Disease-inducing miRNAs can be persistently silenced in vivo through systemic delivery of miRNA inhibitors, allowing for therapeutic modulation of disease mechanisms. Opportunities for manipulating miRNA biology in the settings of muscle disease will be discussed.

Long-term, efficient inhibition of miRNA function in mice using Tough Decoys delivered by adeno-associated virus vectors. Phillip D. Zamore. University of Massachusetts Medical School, Worcester, MA.

Understanding the function of individual miRNA species in mice would require the production of hundreds of loss-of-function strains. To accelerate analysis of miRNA biology in mammals, we combined recombinant adeno-associated virus (rAAV) vectors with miRNA 'Tough Decoys' (TuDs) to inhibit specific miRNAs. Intravenous injection of rAAV9 expressing anti-miR-122 or anti-let-7 TuD depleted the corresponding miRNA and increased its mRNA targets. rAAV producing anti-miR-122—but not anti-let-7—TuD reduced serum cholesterol by 40% for 18 weeks in wild-type mice. High throughput sequencing of liver miRNAs from the treated mice confirmed that the targeted miRNAs were depleted and revealed that TuD RNAs induce miRNA tailing and trimming in vivo. rAAV-mediated miRNA inhibition thus provides a simple way to study miRNA function in adult mammals and a potential therapy for dyslipidemia and other diseases caused by miRNA deregulation.

Session 6: miRNA Regulation in Cancer

microRNAs as therapeutic targets. Carlo M. Croce. The Ohio State University, Columbus, Ohio.

Chronic lymphocytic leukemia (CLL) is the most common leukemia among adults in the Western world, with an annual incidence in the United States of approximately 10 000 new cases. The clinical staging systems devised by Rai et al. and Binet et al. are useful for assessing the extent of CLL in a patient, but they fail to differentiate between the indolent and aggressive forms of CLL. Most typically these forms are characterized by low and high levels of zeta-chain (TCR)–associated protein kinase 70kDa (ZAP70), respectively. Using fluorescence in situ hybridization (FISH), Döhner et al. found that chromosomal abnormalities occurred in 82% of cases and included the 13q deletion (55%), 11q deletion (18%), and 17p deletion (7%). Patients with the 17p and 11q deletions experience the aggressive form of the disease, whereas patients with the 13q deletion or with normal cytogenetic profiles experience the indolent form. The occurrence of common and recurring chromosomal abnormalities suggests that these deletions affect thus-far undefined pathways important for the pathogenesis of CLL.

microRNAs are small, noncoding RNAs with regulatory functions7; microRNA expression is frequently deregulated in tumors. In most CLLs, the expression of the microRNA 15a (miR-15a [GenBank 406948])/microRNA 16-1 (miR-16-1 [GenBank 406950]) cluster, which maps within a 30-kilobase region of loss at 13q14.3 (henceforth designated 13q14), is abolished or reduced. We have previously shown that the expression of this microRNA cluster is inversely correlated with the expression of B-cell CLL/lymphoma 2 (BCL2 [GenBank 596]), an anti apoptotic gene overexpressed in most CLLs. The loss of the long arm of chromosome 11 involves the 11q23.1 region (henceforth designated 11q23), where the microRNA 34b (miR-34b [GenBank 407041]) /microRNA 34c (miR-34c [GenBank 407042]) cluster is located.13 The observation that microRNA 34a (miR-34a [GenBank 407040]), miR-34b, and miR-34c are transactivated by tumor protein p53 (TP53)14 and that the miR-34b/miR-34c cluster maps at 17p suggests the possible existence of a genetic link and significant molecular interactions between the 17p and 11q chromosomal deletions in CLL. At present, it is not known how the 13q, 11q, and 17p deletions contribute to CLL pathogenesis and affect the outcome of patients with CLL.

microRNA reprogramming in cancer: Mechanisms and consequences. Joshua T. Mendell. UT Southwestern Medical Center, Dallas, TX.

miRNA gain- and loss-of-function can potently influence cellular behavior in normal physiologic states and in diseases such as cancer. The regulation of miRNA expression and activity by cellular signaling cascades can therefore result in dramatic phenotypic outputs. We previously demonstrated extensive control of miRNA expression by well-characterized oncogenic and tumor suppressor networks including the Myc, Kras, and p53 pathways. We are now employing novel mouse models with gain and loss of miRNA function to investigate the roles of the miRNAs embedded within these signaling pathways and the pathologic consequences when their functions are disrupted. Insights gained from these functional studies have led to the development of novel therapeutic strategies for cancer and other pathologic states based on miRNA delivery. I will present our latest findings related to miRNA regulation in cancer and exploitation of these findings for the development of novel therapeutic approaches.

The ceRNA hypothesis and the noncoding revolution in cancer research and therapy. Pier Paolo Pandolfi. Beth Israel Deaconess Cancer Center and Harvard Medical School, Boston, MA.

The central dogma of molecular biology, as proposed by Francis Crick, demonstrates that genetic information is transferred from DNA in our genomes to the generation of functional proteins, through a messenger RNA (mRNA) intermediate. This suggests that the key function of each mRNA is to encode for protein.

We have demonstrated that mRNAs can exert a biological activity that is independent of the protein for which they encode. Since this phenomenon is dependent on sequences contained in RNA transcripts, this phenomenon applies to protein-coding genes, pseudogenes, of which 19,000 have been predicted in the genome as well as long non-coding RNAs that have been recently identified to be pervasive in the cell and in biology.

This new function is brought about by the ability of mRNAs—and RNAs in general—to bind and sequester microRNA molecules. microRNAs specifically repress the expression levels of many genes and have consequently been shown to play important roles in diseases including cancer. We show that any messenger RNA and RNA molecule can sequester microRNA molecules acting as a "competitive endogenous RNAs." We therefore term them *ceRNAs*. Given that microRNAs can bind to multiple mRNAs, we argued that mRNAs compete for the binding to a given microRNA. We originally tested this hypothesis and proved its validity by studying the interaction between the mRNA encoding for the *PTEN* tumor suppressor gene and its closely related pseudogene, *PTENP1*, which we show to act as a tumor suppressor through this new mechanism. We therefore identify PTENP1 as well as the several thousand uncharacterized RNA molecules as potential human disease genes.

We have now expanded this analysis to the whole transcriptome. Our findings therefore defines a new biological dimension that will allow for the rapid identification and functional characterization of new disease genes.

Session 7: RNA in Cancer and Disease

Functional genomics, small RNAs, and cancer. William C. Hahn. Dana-Farber Cancer Institute, Boston, MA.

Efforts to sequence cancer genomes have begun to uncover comprehensive lists of genes altered in cancer. Unfortunately, the complexity and sheer volume of data that emerges from these efforts has made dissecting the underlying biology of cancer difficult. In addition, the number and types of candidate targets that require functional validation in vivo represent a significant barrier to using this information as a starting point for drug development programs, since many do not represent targets amenable to traditional small molecule or antibody-based therapeutic approaches. To address this problem, we have combined comprehensive analyses of cancer genomes with novel materials science to develop a platform that enables the ready in vivo validation of cancer targets. Specifically, we have developed a screening platform to perform loss of function screens at genome scale and have applied this in a massively parallel manner to screen several hundred full characterized cancer cell lines. We have combined the outputs of these screens with data emerging from the study of cancer genomes to identify candidate oncogenes. To credential these targets in vivo, we have helped develop novel nanocomplexes formed from siRNA bound to tandem peptides containing tumor-penetrating, endosomal escape and siRNA-binding domains that permit deep penetration in tumor. We use these nanoparticles to target a hitherto undiscovered ovarian cancer oncogene, ID4, an undruggable transcriptional regulator, and show that these tumor-penetrating nanocomplexes induced complete suppression of tumor growth and significant improvement of survival. Taken together, these studies provide a platform for the discovery and initial validation of candidate cancer targets.

Mechanisms of miRNA-mediated cellular reprogramming. Edward Morrisey. University of Pennsylvania, Philadelphia, PA.

Recent evidence has indicated that miRNAs have the ability to promote dramatic changes in cell fate including pluripotent stem cell reprogramming. Certain miRNA clusters including miR302/367 are highly expressed in embryonic stem cells and when over-expressed can convert fibroblasts to induced pluripotent stem cells. Moreover, recent publications have shown that certain miRNAs can directly convert fibroblasts to neurons indicating that this technique may be widely applicable to other cell lineages. Despite

these intriguing results, little is understood about the molecular mechanism underlying miRNA mediated reprogramming. Since miRNAs can target hundreds of mRNAs, identification of specific targets as well as molecular pathways affected will require multiple approaches. Data will be presented on how our lab is using systems biology and genomic approaches to decipher the underlying mechanism of miRNA-mediated cellular reprogramming.

New therapeutic strategies for lymphomas inspired by functional and structural genomics. Louis M. Staudt. National Cancer Institute, Bethesda, MD.

Seeking an unbiased method to discover therapeutic targets in cancer, we developed a loss-of-function genetic screen using genomic-scale libraries of small hairpin RNAs that mediate RNA interference. These "Achilles heel" screens are designed to reveal genes essential for cancer cell proliferation and survival. In a parallel structural genomics approach, we are using RNA-seq to globally identify somatic mutations and other structural abnormalities in cancer. RNA-seq also provides an unprecedented view of gene expression that provides more accurate quantitative data than traditional microarray approaches. The intersection of the RNA interference and RNA-seq data sets has helped us to discover novel pathogenetic pathways that control the proliferation and survival of the aggressive lymphoma subtypes. The malignant phenotype is seen as an aberrant regulatory network that includes oncogene-induced microRNAs, deregulated transcription factors and aberrant signaling from cell surface receptors. This view suggests several therapeutic opportunities using targeted agents that might initially augment and eventually supplant the more toxic chemotherapeutic regimens used to treat these cancers today.

Session 8: RNA Therapeutics

Targeting of microRNAs for therapeutics. Sakari Kauppinen. Santaris Pharma, Horsholm, Denmark.

microRNAs act as important post-transcriptional regulators of gene expression by mediating mRNA degradation and translational repression. Moreover, there is ample evidence that perturbations in the levels of individual or entire families of miRNAs are strongly associated with the pathogenesis of a wide range of human diseases. Besides cancer, miRNAs have also been implicated in viral infections, cardiovascular and muscle diseases and CNS disorders. Thus, disease-associated miRNAs represent a potential new class of targets for oligonucleotide-based therapeutics, which may yield patient benefits unobtainable by other therapeutic approaches.

LNA is a bicyclic high-affinity RNA analogue, in which the ribose ring is locked in an RNA-like, N-type (C3'-endo) conformation by the introduction of a 2'-O,4'-C methylene bridge. Transfection of LNA-modified antimiR oligonucleotides into cells results in potent and specific inhibition of miRNA function with concomitant derepression of direct target mRNAs. In addition, systemically delivered, unconjugated LNA-antimiRs with a phosphorothioate backbone show high metabolic stability and uptake in many tissues in mice, coinciding with long-term miRNA silencing in vivo. These findings support the utility of LNA-modified antimiR oligonucleotides in the development of therapeutic strategies aimed at pharmacological inhibition of disease-associated miRNAs. We will describe recent progress in targeting of disease-implicated miRNAs for therapeutics using LNA-antimiRs and present an update on the clinical development of antimiR-122 for treatment of hepatitis C virus infection.

Long noncoding RNAs as drug targets.
Claes Robert Wahlestedt. University of Miami Miller School of Medicine, Miami, FL.

Much of the mammalian genome is transcribed into non-coding RNAs of different categories. This lecture will primarily be concerned with natural antisense transcripts (NATs) most of which are long noncoding RNAs. NATs are found in most gene loci and regulate gene expression through several distinct mechanisms including chromatin modifications. Inhibition/ perturbation of endogenous NATs by modified oligonucleotides called AntagoNATs, in vitro or in vivo, reveals concordant or discordant regulation and results in down- or up-regulation of conventional (protein-coding) gene expression, respectively. Evidence will be presented that AntagoNATs can potently induce locus-specific and reversible modulation of gene expression associated with alterations in chromatin marks.

Proffered Abstracts

PR1 BRAF^{V600E} remodels the melanocyte transcriptome and induces BLNCR1 to regulate melanoma cell migration. Ross J. Flockhart, Dan E. Webster, Kun Qu, Nicholas Mascarenhas, Joanna Kovalski, Markus Kretz, Paul A. Khavari. Stanford University, Stanford, CA.

Aberrations of protein-coding genes are a focus of cancer genomics, however, the impact of oncogenes on expression of the ~50% of transcripts without protein-coding potential, including long non-coding RNAs (lncRNAs), is largely uncharacterized. Activating mutations in the BRAF oncogene are present in 60% of melanomas, 90% of which produce active mutant BRAF^{V600E}. To define the impacts of oncogenic BRAF on the melanocyte transcriptome, massively parallel cDNA sequencing (RNA-Seq) was performed on genetically matched normal human melanocytes with and without BRAF^{V600E} expression. To enhance potential disease relevance by verifying expression of altered genes in BRAF-driven cancer tissue, parallel RNA-Seq was also undertaken on two BRAF^{V600E}-mutant human melanomas. BRAF^{V600E} regulated expression of 1,027 protein-coding transcripts and 39 annotated lncRNAs, as well as 70 un-annotated, potentially novel, intergenic transcripts. Coding potential analysis of these 70 transcripts suggested that most may represent newly identified non-coding RNAs. BRAF-regulated long non-coding RNA 1 (BLNCR1) was identified as a recurrently, highly expressed, previously un-annotated 693 bp transcript on chromosome 9 with a potential functional role in melanoma cell migration. Combining RNA-Seq of oncogene-expressing normal cells with RNA-Seq of their corresponding human cancers may represent a useful approach to discover new oncogene-regulated RNA transcripts of potential clinical relevance to cancer.

This abstract is also presented as Poster A11.

PR2 Functional characterization of p53-regulated long intervening noncoding RNAs (lincRNAs). Nadya M. Dimitrova, Tyler Jacks. David H. Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA.

LincRNAs (long intervening noncoding RNAs) constitute a novel class of long, noncoding RNAs that have been implicated in regulating gene expression in diverse biological processes by modulating the activity of chromatin-modifying complexes. In collaboration with the Rinn laboratory at the Broad Institute, we have identified a set of lincRNAs that appear to be direct transcriptional targets of p53. These lincRNAs contain p53 binding elements in their promoter regions and are strongly upregulated in a p53-dependent manner upon DNA damage and in response to oncogenic stress. We have hypothesized that these lincRNAs may be new components of the p53 network and may contribute to p53 tumor suppressor function by regulating the expression of genes in cancer-relevant pathways. Using a combination of molecular genetic studies, cell biology, biochemistry, as well as gene targeting in the mouse, our aim is to dissect the biological function of these lincRNAs in vivo and in isolated cells.

To date, we have generated a conditional knockout mutant for lincRNA-p21, one of the p53-regulated lincRNAs, which was recently shown to mediate gene repression in the p53 transcriptional network. Using this genetic tool, we plan to characterize the consequences of lincRNA-p21 loss in mice and in isolated mouse embryonic fibroblasts. Our goal is to identify genes that are regulated by lincRNA-p21 and to map out interactions between lincRNA-p21 and the p53 transcriptional network. We will also focus on dissecting the mechanism by which lincRNA-p21 may regulate the expression of target genes.

We have also identified a lincRNA, linc9, which is induced by DNA damage in a dose-dependent manner. Even though the expression of linc9 appears to be p53-

independent, our preliminary experiments indicate that linc9 may influence the outcome of p53 activation by promoting apoptosis in response to high levels of DNA damage.

In sum, by studying the function and mechanism of p53-regulated and DNA damage-induced lincRNAs, we hope to achieve a better understanding of the role of long noncoding RNAs in modulating the p53 pathway and more broadly in regulating cancer-related processes.

This abstract is also presented as Poster B19.

PR3 Characterization of a novel pseudogene expressed antisense RNA that regulates PTEN expression. Per Johnsson[1], Amanda Ackley[2], Dan Grandér[1], Kevin V. Morris[2]. [1]Karolinska Institutet, Stockholm, Sweden, [2]The Scripps Research Institute, La Jolla, CA.

PTEN is a tumor suppressor gene that is dysregulated in several different forms of cancer. PTEN was recently reported to be involved in a regulatory microRNA network where the PTEN pseudogene (PTENpg1) sequesters microRNAs and thus affects the translation of the PTEN mRNA.

Here, we show that the pseudogene-based regulation of PTEN is more complex than previously appreciated. We report that the PTENpg1 locus also encodes an antisense RNA (asRNA), which directly regulates PTEN on the transcriptional level in trans. We show that the PTENpg1 asRNA localizes to the PTEN promoter and also binds to the DNA methyltransferase 3a (DNMT3a). Overexpression of the PTENpg1 asRNA suppresses the expression of PTEN while depletion of the PTENpg1 asRNA decreases DNMT3a and EZH2 mediated epigenetic silencing of the PTEN promoter, resulting in increased transcriptional activity of PTEN. We further show that specific targeting of the PTENpg1 asRNA induces G0/G1 cell cycle arrest and sensitizes relatively chemoresistant cells to DNA damaging doxorubicin treatment.

We also find that the PTENpg1 asRNA functions as a concordant regulator of the PTENpg1 sense by stabilizing this transcript via an RNA:RNA interaction. This RNA:RNA interaction stabilizes the PTENpg1 sense transcript as well as its potential to sequester microRNAs and PTEN mRNA translation.

In summary, we have characterized a novel PTENpg1 encoded asRNA that acts on the PTEN gene by recruitment of chromatin-remodeling factors such as DNMT3a and EZH2 to the PTEN promoter. We also demonstrate that PTENpg1 asRNA functions as a dual-type regulator of both PTEN transcription and translation. Knowledge of this molecular pathway may prove useful in understanding epigenetic and asRNA modulation of gene expression in human cells, and in the case of PTEN may prove beneficial in developing targeted approaches to induce PTEN transcription.

This abstract is also presented as Poster B25.

PR4 Molecular function of the RNA binding protein EWS in RNA processing. Bethsaida I. Nieves, Shuang Niu, Dedeepya Vaka, Julia Salzman, Patrick Brown, E. Alejandro Sweet-Cordero. Stanford University School of Medicine, Stanford, CA.

The Ewing sarcoma (EWS) protein is a member of the FET family of RNA-binding proteins and is involved in chromosomal translocations that generate oncogenic fusion genes found in a variety of sarcomas. EWS has been recently reported to be involved in regulating transcription, pre-mRNA processing, translation and DNA repair. However, the precise physiological role of EWS in RNA processing remains largely unknown. Using cross-linking immunopercipitation coupled with high-throughput sequencing (CLIP-Seq), we identified the full complement of RNAs bound to EWS in Hela cells. Analysis of RNA binding by EWS revealed that the greatest increase in binding was to A-I editing enzymes ADAR1, ADARB1 and ADARB2 as well as long non-coding RNAs (lncRNAs) NEAT1 and MALAT1. Interestingly, these lncRNAs localize to specific subnuclear structures and have been implicated in regulating A-I editing and RNA splicing respectively. We hypothesize that EWS could play a role in regulating the subnuclear localization of other proteins involved in editing and splicing by virtue of its association with NEAT1 and

MALAT1. We also found that binding of EWS to pre-mRNAs influenced alternative splicing. Paired-end RNA-Seq of the transcriptome revealed that knock down of EWS resulted in the over expression of 116 genes and the down regulation of 68 genes, including EWS itself. Further analysis identified 16 alternatively spliced genes following EWS knock down across two independent RNA seq experiments. Of these genes, 6 were also shown to bind EWS by CLIP-seq. These genes include, CENPA, CSTF1, RBBP6, RAC1, FANC1 and ANAPC5. These studies highlight a potential role for EWS as a critical regulator of alternative splicing and A-I editing.

This abstract is also presented as Poster B8.

PR5 Treatment of ovarian cancer with targeted tumor-penetrating siRNA nanocomplexes. Yin Ren[1], Hiu Wing Cheung[2], Ronny Drapkin[3], David Root[2], Justin Lo[4], Valentina Fogal[5], Erkki Ruoslahti[5], William Hahn[2], Sangeeta Bhatia[4], Geoffrey von Maltzahn[1], Amit Agrawal[1], Glenn Cowley[2], Barbara Weir[2], Jesse Boehm[2], Pablo Tamayo[2], Jill Mesirov[2], Alison Karst[3]. [1]Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA, [2]Broad Institute of Harvard and MIT, Cambridge, MA, [3]Dana-Farber Cancer Institute, Boston, MA, [4]Massachusetts Institute of Technology, Cambridge, MA, [5]Burnham Institute for Medical Research, Santa Barbara, CA.

Whole-genome analysis of cancer samples is identifying many potential therapeutic targets, by virtue of their being frequently mutated or functionally essential in specific types of cancer. However, we lack efficient ways to test the therapeutic benefit of modulating targets in vivo. RNAi offers one potential solution; however, approaches to deliver siRNA in vivo have been challenging due to their susceptibility to serum nucleases, endosomal entrapment, and stimulation of innate immunity. Furthermore, nanoparticle- and antibody-based siRNA delivery approaches have historically suffered from limited tumor penetration and low transvascular transit, thereby limiting the applicability of parenchymal siRNA targets. Here we describe a tumor penetrating nanocomplex (TPN) comprised of siRNA complexed to a tandem tumor-penetrating and membrane-translocating peptide, which enables the homing of siRNA deep into tumor parenchyma. Upon complexation with siRNA, the resulting nanocomplex is stable, non-immunostimulatory, displays homing peptides in a multivalent fashion that increases their binding avidity and delivers siRNA to the cytosol of tumor cells through receptor-specific interactions and membrane translocation. Upon systemic administration into mice, this nanocomplex penetrates into the parenchyma of metastatic peritoneal tumors and silences target genes in cells of interest in a receptor-specific manner. We employed TPNs in vivo to evaluate ID4, a novel candidate oncogene in ovarian cancer, which we identified by combining genome-scale RNAi screening of cancer cell lines with genome-scale sequence analysis of patient tumors. We show that treatment of tumor-bearing mice with ID4-specific TPNs suppresses tumor growth and significantly improved survival. These findings provide a framework for the identification, credentialing, and understanding of novel cancer targets as well as validating a specific therapeutic target in ovarian cancer.

This abstract is also presented as Poster B2.

Poster Session A

A1 Long stress-induced noncoding transcripts (LSINCTs) and cancer. David I. Smith[1], Silva M. Jessica[2]. [1]Mayo Clinic, Rochester, MN, [2]Washington University, St. Louis, MO.

We previously utilized a whole genome tiling array to identify novel transcripts which had altered expression in response to the genotoxic effects of the tobacco carcinogen NNK. Our goal was to identify novel long stress-induced non-coding transcripts that could play important regulatory roles within cells and be important targets of alteration during cancer formation. Utilizing very stringent criteria, we examined all transcriptionally active regions that had altered expression in response to NNK treatment and identified a group of 12 transcripts, termed long stress-induced noncoding trancripts (LSINCTs). These novel long non-coding transcripts are between 2 and 4 Kb in length and do not contain any open reading frames or have homology to any mRNAs. 11 LSINCTs are intergenically located and one LSINCT is intragenically located within the genome. We found LSINCTs to be more highly expressed in more proliferative normal human tissues and to be frequently over-expressed in a number of different cancers including breast cancer. We studied one of these transcripts in greater detail, LSINCT5. We found LSINCT5 to be a polyadenylated nuclear transcript of exactly 2,647 base pairs. It is highly over-expressed in both breast and ovarian cancers and knocking down its' expression in either a breast or ovarian cancer cell line resulted in decreased cellular proliferation. We further compared gene expression in the breast cancer cell line, MCF7, before and after knocking down LSINCT5 expression and identified a number of genes whose expression changed more than 2-fold in response. Two of the genes identified were the gene for the major paraspeckle protein PSPC1, and another long non-coding transcript NEAT1. Paraspeckles are irregularly shaped compartments found in the nucleus' interchromatin space. Paraspeckles are RNA-protein structures formed by the interaction of NEAT1 and members of the Drosophila Behavior Human Splicing family of proteins including PSPC1. Paraspeckles appear to be critical to the control of gene expression through the nuclear retention of certain species of RNA. It is though that paraspeckles and their components play a role in controlling gene expression during a number of different cellular processes including differentiation, viral infection and stress responses. Using RNA fluorescent in situ hybridization, we found LSINCT5 to co-localize with NEAT1 within the nucleus. Hence, this novel stress-induced transcript may be another important long non-coding transcript that is involved in the normal cellular function of the paraspeckles.

A2 miR-10a regulation of PIK3CA and response to Taxol in non-small cell lung cancer. Chris DeSevo[1], Liqin Du[2], Carmen Behrens[3], Ignacio I. Wistuba[3], John Minna[1], Alexander Pertsemlidis[2]. [1]UT Southwestern Medical Center at Dallas, Dallas, TX, [2]UT Health Science Center at San Antonio, San Antonio, TX, [3]UT MD Anderson Cancer Center, Houston, TX.

Phosphatidylinositol 3-kinases (PI3Ks) are a family of enzymes involved in diverse cellular functions including cell growth, proliferation, differentiation, motility, survival and apoptosis. PI3K allows for the transmission of extracellular cues to an intracellular response via its activation through receptor tyrosine kinase or k-ras. Activation of this pathway is antagonized by the phosphatase PTEN. This signaling pathway is under extremely tight regulation and even slight perturbations can lead to aberrant pathway activation, as is the case in a majority of cancers. We are interested in whether PIK3CA is regulated by one or more microRNAs and whether those miRNAs have a therapeutic effect on cancer cell viability and drug response in PI3K-driven oncogenesis.

In a systematic approach to identifying miRNA inhibitors with significant effects on cell

viability and response to paclitaxel in NSCLC, we performed a high-throughput screen and identified several candidate microRNAs. Regulatory targets of candidate miRNAs were identified through a combination of in vitro and in silico approaches. Targets were then validated using qRT-PCR, protein quantification, and luciferase reporter assays. The response of cancer cells to perturbations of the candidate miRNA was assessed through flow cytometric analysis of cell cycle phase distribution, colony formation and caspase activation assays.

The inhibitor screen revealed that inhibition of miR-10a increased cellular growth rate and resistance to paclitaxel. Further validation demonstrated a 10-fold increase in cell viability in the presence of miR-10a inhibitor, and a 10-fold decrease in the presence of miR-10a mimic. Manipulation of miR-10a levels in either direction resulted in significant changes in both mRNA and protein levels of its predicted target, the catalytic subunit of phosphatidylinositol 3-kinase (PI3K), which was confirmed to be a direct target by luciferase reporter assay. Increasing miR-10a levels inhibited the ability to form colonies, while decreasing miR-10a levels increased colony formation. To assess the prognostic value of miR-10a we assessed expression in NSCLC patients and observed that high miR-10a levels correlate with longer overall survival.

The identification of a miR-10a as a modulator of cellular response to taxanes, and PI3K as a regulatory target which mediates that response, define a novel regulatory pathway modulating paclitaxel sensitivity in lung cancer, which may provide novel adjuvant strategies along with paclitaxel in the treatment of lung cancer and may also provide biomarkers for predicting paclitaxel response in NSCLC. Ultimately this study will provide new insights into the molecular basis of lung cancer and a new set of potential therapeutic targets for this devastating disease.

A3 miR-31 and its host gene lncRNA LOC554202 are regulated by promoter hypermethylation in triple-negative breast cancer. Katarzyna Augoff, Brian McCue, Edward F. Plow, Khalid Sossey-Alaoui. Cleveland Clinic Lerner Research Institute, Cleveland, OH.

Background: microRNAs have been established as powerful regulators of gene expression in normal physiological as well as in pathological conditions, including cancer progression and metastasis. Recent studies have demonstrated a key role of miR-31 in the progression and metastasis of breast cancer. Downregulation of miR-31 enhances several steps of the invasion-metastasis cascade in breast cancer, i.e., local invasion, extravasation and survival in the circulation system, and metastatic colonization of distant sites. miR-31 exerts its metastasis-suppressor activity by targeting a cohort of pro-metastatic genes, including RhoA and WAVE3. The molecular mechanisms that lead to the loss of miR-31 and the activation of its pro-metastatic target genes during these specific steps of the invasion-metastasis cascade are however unknown.

Results: In the present report, we identify promoter hypermethylation as one of the major mechanisms for silencing miR-31 in breast cancer, and in the triple-negative breast cancer (TNBC) cell lines of basal subtype, in particular. miR-31 maps to the intronic sequence of a novel long non-coding (lnc)RNA, LOC554202 and the regulation of its transcriptional activity is under control of LOC554202. Both miR-31 and the host gene LOC554202 are down-regulated in the TNBC cell lines of basal subtype and over-expressed in the luminal counterparts. Treatment of the TNBC cell lines with either a de-methylating agent alone or in combination with a de-acetylating agent resulted in a significant increase of both miR-31 and its host gene, suggesting an epigenetic mechanism for the silencing of these two genes by promoter hypermethylation. Finally, both methylation-specific PCR and sequencing of bisulfite-converted DNA demonstrated that the LOC554202 promoter-associated CpG island is heavily methylated in the TNBC cell lines and hypomethylated in the luminal subtypes.

Conclusion: Loss of miR-31 expression in TNBC cell lines is attributed to hypermethylation of its promoter-associated CpG island. Together, our results provide the initial evidence for a mechanism by which miR-31, an important determinant of the invasion metastasis cascade, is regulated in breast cancer.

A4 The functional role of the PCA3 noncoding RNA in human prostate cancer. Gerald W. Verhaegh, Kelly van den Oudenalder, Frank Smit, Martijn A. Huynen, Jack A. Schalken. Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Introduction: *PCA3* is a prostate-specific gene that is highly over-expressed in prostate cancer. The *PCA3* gene lacks an extensive open reading frame and no in vitro translation products could be identified, suggesting that *PCA3* acts as a non-coding RNA (ncRNA). The aim of this study is to reveal the function of the *PCA3* ncRNA and its mechanism.

Material and Methods: To study the phenotypic effects of *PCA3*, prostate cancer cell lines were transfected with *PCA3* expression vectors. Anchorage-independent cell growth was measured by colony formation in soft agar. *PCA3* RNA-protein interactions were studied by sucrose gradient centrifugation and by protein-RNA UV-cross-linking assays. In silico PCA3 RNA secondary structure modeling was performed using the Vienna RNA Package (v.1.6). MicroRNA processing and expression were determined by Northern blotting, AGO2 RNA co-IP, and qPCR analysis.

Results: *PCA3* over-expressing prostate cancer cells displayed an enhanced capacity to form colonies in soft agar (i.e., more and bigger colonies were formed). This finding indicates that *PCA3* plays a role in prostate cancer cell proliferation and survival. After sucrose centrifugation of total cell extracts, *PCA3* was present in fractions of higher density compared to naked RNA, indicating that *PCA3* RNA-protein binding occurs. Indeed, in UV cross-linking assays, the *PCA3* RNA specifically interacted with several low molecular-weight proteins. In silico analysis of the *PCA3* transcript revealed a number of stable secondary RNA structures that may be precursors for microRNA biogenesis. On Northern blots, one small *PCA3*-derived RNA could be detected, and this small *PCA3* RNA was found to be in complex with the AGO2 protein. MiRNA qPCR analysis revealed that this *PCA3*-derived miRNA was up-regulated in prostate cancer.

Discussion: Our results demonstrate that *PCA3* contributes to prostate cancer proliferation and survival. The formation of specific *PCA3* RNA-protein complexes and the processing of the PCA3 transcript into a microRNA may contribute to the observed *PCA3*-mediated growth stimulation. Identification of the *PCA3* RNA-binding proteins and *PCA3* microRNA target genes is ongoing.

A5 Cytotoxic extracts of ethnomedicinal plants: *Podophyllum peltatum* (mayapple) and *Echinacea angustifolia*. Olena T. James, E. Lewis Myles. Tennessee State University, Nashville, TN.

Phytochemical and other natural compounds are historically established as cures for many ailments in rural America and other developing countries. Our lab is testing plants that show potential for inhibiting the growth of cancer cells. The family Asteraceae and the family Berberidacae are very well known for their values as medicinal plants. More specifically, the genus species Echinacea angustifolia and genus species Podophyllum peltatum commonly known as the Mayapple, both are noted for their unique medicinal purposes. The crude methanol extracts of these plants were tested against human metastatic cell lines representing breast (MCF-7), (BT-549) and colon (SW620) tissues. Trypan blue exclusion is used to determine viable cell counts. Alamar BlueTM and florescent analysis were used to evaluate the cytoxicity of the extracts. Analysis of the crude extracts of Echinacea angustifolia has shown on MCF-7, BT-549, and SW620 that most of them exhibit anticancer activity against the cell lines at the maximum concentration of 200 µg /mL. In particular, less diluted concentrations have demonstrated exceptional inhibitory effects

with IC50 values ranging between 0.10-15 µg /mL, respectively. On the other hand, analysis of the crude extracts of Podophyllum peltatum (Mayapple) has shown on MCF-7, BT-549 and SW620 some anticancer activity against cell lines at the maximum concentration of 200 µg /mL. More specifically, the more diluted concentrations have demonstrated exceptional growth inhibition with IC50 values ranging between 2-8 µg /mL

A6 microRNA sequencing of AKXD recombinant inbred panel identifies miR-216b as a candidate metastasis suppressor in a murine model of breast cancer. Farhoud Faraji[1], Ying Hu[1], Natalie Goldberger[1], Gang Wu[2], Ken H. Buetow[1], Jinghui Zhang[2], Kent W. Hunter[1]. [1]National Cancer Institute, Bethesda, MD, [2]St. Jude Children's Research Hospital, Memphis, TN.

Metastatic disease continues to be the primary cause of mortality in cancer. Using a combination of complex genetics, mouse models of breast cancer, and human epidemiology, our laboratory has demonstrated that germline polymorphism contributes to an individual's susceptibility to metastasis. In the current study, we have used the AKXD recombinant inbred panel, which is derived from inbred mice of metastasis resistant, DBA, and metastasis-prone, AKR, genetic backgrounds, to identify microRNAs whose expression is associated with metastasis. microRNA sequencing of tumor tissue from the progeny of AKXD x Polyoma Middle-T mice demonstrated that the expression of the miR-216/217 cluster is significantly negatively correlated with pulmonary metastases in the AKXD panel. Linkage analysis demonstrated a significant association between the miR-216/217 locus and the DBA allele, suggesting that the difference in expression is inherited rather than somatically acquired. Finally, we show that ectopic expression of miR-216b in murine mammary tumor cells suppresses pulmonary metastasis without significantly impacting primary tumor growth in vivo. These findings suggest that miR-216b is an inherited modifier of metastasis with metastasis suppressing activity in a mouse model of breast cancer.

A7 Roles of miRNAs in the switch of TGF-β from tumor suppressor to pro-oncogenic factor in cancer progression. Yoshiko Nagano, Maxwell P. Lee, Lalage M. Wakefield. National Cancer Institute, Bethesda, MD.

TGF-β is a widely expressed pleiotropic growth factor that plays complex roles in tumorigenesis. TGF-β switches from tumor suppressor to pro-oncogenic factor during breast cancer progression. Our goal is to identify molecular determinants of the TGF-β switch. Since miRNA expression is deregulated in cancer, and TGF-β is reported to regulate expression and biogenesis of miRNAs, we hypothesize that miRNAs may contribute to this switch. To model the TGF-β switch, we used a series of human breast cancer cell lines derived from the spontaneously immortalized MCF10A human breast epithelial cell line. The model consists of 4 cell lines. In the MCF10A (M-I; normal), MCF10AT (M-II; premalignant) and MCF10CA1hcl4 (M-IIIcl4; low-grade tumor) lines, TGF-β is a tumor suppressor. In contrast, in the MCF10CA1a cell line (M-IV), which gives rise to aggressive metastatic tumors, the tumor suppressor activity is lost and TGF-β promotes metastasis. To test the possible involvement of miRNAs in the TGF-β switch, we compared expression profiles of miRNAs by Next Gen miRNA sequencing 1) basally in cultures of all four cell lines and 2) in M-IIIcl4 and M-IV with and without TGF-β treatment. A variety of patterns of change in basal miRNA expression with malignant progression were seen. We focused on miRNAs whose expression level differs basally between M-IIIcl4 and M-IV, since the switch occurs between these two cell lines. 46 miRNAs were expressed > 1.5 fold higher in M-IIIcl4 than in M-IV, whereas 29 miRNAs were expressed > 1.5 fold higher in M-IV than in M-IIIcl4. We selected several miRNAs whose expression was most different between the two cell lines, and examined whether these miRNAs regulate cellular responses to TGF-β. As an example, let-7i and let-7g were expressed higher in M-IV than in M-IIIcl4. Forced expression of these miRNAs partially reversed TGF-β-induced growth inhibition in M-IIIcl4. A similar strategy was applied to miRNAs whose regulation

by TGF-β is different between M-IIIcl4 and M-IV. The results of these experiments will be discussed.

A8 Defining specific microRNAs that cooperatively regulate GRP78, an ER chaperone and signaling regulator, in cancer. Sheng-Fang Su, Amy S. Lee, Gangning Liang. USC/Norris Comprehensive Cancer Center, Los Angeles, CA.

GRP78, a major endoplasmic reticulum (ER) chaperone and signaling regulator, is commonly over-expressed in cancer and contributes to cancer cell proliferation, survival and tumorigenesis. Moreover, induction of GRP78 by a variety of anti-cancer drugs, including histone deacetylase inhibitors, confers chemo-resistance to cancer. Thus, targeting GRP78 to inhibit tumor survival and proliferation, sensitize tumor cells to chemotherapeutic drugs and consequently induce apoptosis serves as a promising avenue in anti-cancer therapy. As endogenous regulators, microRNAs (miRNAs) play important roles in modulating gene expression; therefore, we sought to identify miRNA(s) that target GRP78. We report that multiple miRNAs (miR-30d, miR-181a, miR-199-5p) commonly down-regulated in human cancer cell lines can repress GRP78 and lead to increased apoptosis in C42B prostate cancer cells. Luciferase reporter assays indicate that these miRNAs act as negative regulators of GRP78 by directly targeting the 3' untranslated region of GRP78. Importantly, the combination of multiple miRNAs cooperated resulting in greater inhibition on GRP78 than individual miRNAs, suggesting the efficacy of tumor suppression by combined miRNAs. In addition, we showed that transfection of the multiple miRNAs in C42B cells increased sensitivity of cancer cells to trichostatin A (TSA), a histone deacetylase inhibitor, induced apoptosis, and inhibited cell proliferation and colony formation. Therefore, we suggest that delivery of combinations of specific miRNAs could be a novel anti-cancer approach capable of suppressing GRP78 and in turn inhibiting GRP78-mediated tumorigenesis.

A9 De novo sequencing of circulating microRNAs identifies novel markers predicting clinical outcome of locally advanced breast cancer. Xiwei Wu, George Somlo, Melanie Palomares, Yun Yen, John Rossi, Harry Gao, Shizhen Emily Wang. City of Hope Beckman Research Institute and Medical Center, Duarte, CA.

Purpose: MicroRNAs (miRNAs) have been recently detected in the circulation of cancer patients, where they are associated with clinical parameters. Discovery profiling of circulating small RNAs has not been reported in breast cancer (BC), and was carried out in this study to identify blood-based small RNA markers of BC clinical outcome.

Patients and Methods: The pre-treatment sera of 42 stage II–III locally advanced and inflammatory BC patients who received neoadjuvant chemotherapy (NCT) followed by surgical tumor resection were analyzed for marker identification by deep sequencing all circulating small RNAs. An independent validation cohort of 26 stage II–III BC patients was used to assess the power of identified miRNA markers.

Results: More than 800 miRNA species were detected in the circulation, and exhibited patterns associated with the histopathological profiles of BC. Groups of circulating miRNAs differentially associated with ER/PR/HER2 status and inflammatory BC were identified. The relative levels of selected miRNAs measured by PCR showed consistency with their abundances determined by deep sequencing. Two circulating miRNAs, miR-375 and miR-122, exhibited strong correlations with clinical outcomes, including NCT response and relapse with metastatic disease. In the validation cohort, higher levels of circulating miR-122 specifically predicted metastatic recurrence in stage II–III BC patients.

Conclusion: Our study indicates that certain miRNAs can serve as potential blood-based biomarkers for NCT response, and that miR-122 prevalence in the circulation predicts BC metastasis in early-stage patients. These results may allow optimized chemotherapy treatments and preventive anti-metastasis interventions in future clinical applications.

A10 microRNA 27a is downregulated in cisplatin-resistant bladder cancer cells and contributes to resistance through the targeting of xCT, a cystine transporter involved with glutathione production. Ross M. Drayton, Ewa Dudziec, James W.F. Catto, Helen E. Bryant. Institute for Cancer Studies, Sheffield University Medical School, Sheffield, South Yorkshire, United Kingdom.

Cisplatin resistance represents a serious obstacle to the chemotherapeutic treatment of many tumors. The purpose of this study was to create a cell culture model of cisplatin resistant bladder cancer (CRBC), then use this model to identify changes in the expression of microRNAs (miRs) that may influence resistance.

Single cell clones resistant to cisplatin were generated from parental bladder cancer cell lines by continuous culture in increasing concentrations of cisplatin for several months. The resultant CRBC cell lines were able to tolerate high doses of cisplatin (typically > 10 μM). MicroRNA expression in parental and CRBC cell lines was measured using q-pcr based low-density tiling arrays and a panel of miRs which were consistently altered in all CRBC cell lines was established. In addition the induction and repair of specific cisplatin-DNA adducts in parental and CRBC cell lines was assessed using antibodies against the guanine–guanine (Pt-[GG]) intrastrand crosslink. Four fold more cisplatin was required to produce the equivalent damage in CRBC cell lines compared to the parental control, while the rate of repair, as assessed by measurement of the removal of cisplatin-DNA adducts over time, remained broadly similar between parental and resistant lines. This demonstrated that resistance was due to reduced adduct formation rather than any defect in cross-link repair.

Glutathione (GSH) inactivates cisplatin by binding with it irreversibly to form $Pt(SG)_2$ adducts, thus preventing the drug from forming cytotoxic adducts with DNA. One cause of decreased adduct formation is therefore deregulation of the GSH biosynthesis pathway. Upon examination intracellular levels of GSH and its oxidized form glutathione disulfide (GSSG) were found to be increased in resistant cells. The expression of a number of genes involved with the synthesis of GSH was then measured by rt-pcr and western blotting, and several key proteins involved with GSH biosynthesis were found to be upregulated in resistant cells, suggesting increased levels of intracelluar GSH could be responsible for the observed decrease in the levels of cisplatin-DNA adducts observed in resistant cells.

The 3' untranslated regions (3' UTRs) of these mRNAs were examined using TargetScan, an online miR target prediction program, to find potential sites of miR regulation. The most upregulated protein, xCT (SLC7A11), responsible for cystine import (known to be the rate-limiting step in GSH synthesis) was found to contain target sites for three miRs which had been shown to be significantly downregulated in our model of CRBC, namely miRs 25, 27a and 32.

Upregulation of miRs 25, 27a and 32 by transfection of resistant cells with the pri-miR precursor molecules revealed that restoring expression of miR-27a resensitized CRBC cells to the cytotoxic effects of cisplatin. Restoration of miR-27a expression in CRBC cells was also shown to result in a downregulation of the expression of xCT at both the protein and mRNA level.

In summary, we have identified a panel of microRNAs that are consistently dysregulated in CRBC, and implicated one of these (miR-27a) in the modulation of cisplatin resistance via increased glutathione production. We also provide evidence that reversal of this change in miR expression results in a reversal of the effects on downstream target, and critically, a reversal of the cisplatin resistant phenotype. The contribution made by miR-27a to the development of cisplatin resistance might represent a potential site for therapeutic intervention in the treatment of CRBC. Measurement of miR-27a expression in tumor material or urine may also serve as a predictive biomarker for patients likely response to cisplatin chemotherapy.

A11 BRAFV600E remodels the melanocyte transcriptome and induces BLNCR1 to regulate melanoma cell migration. Ross J. Flockhart, Dan E. Webster, Kun Qu, Nicholas Mascarenhas, Joanna Kovalski, Markus Kretz, Paul A. Khavari. Stanford University, Stanford, CA.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR1) of the conference *Proceedings*.

A12 Identification of microRNA-based therapeutic candidates using a unique lentiviral microRNA overexpression library. Paula I. van Noort[1], Negar Babae[2], Gerald W. Verhaegh[3], Willemijn M. Gommans[1], Francesco Cerisoli[1], Mark Verheul[1], Raymond M. Schiffelers[2], Arjan W. Griffioen[4], Jack A. Schalken[3], Eugene Berezikov[5], Edwin Cuppen[5], Roel Q. J. Schaapveld[1], Jos B. Poell[5], Gregoire P. Prevost[1], Meriem Bourajjaj[1], Suzanna Vidic[1], Judy R. van Beijnum[4], Rick J. van Haastert[1], Iman Schultz[1], Thijs de Gunt[1], Onno van Hooij[3]. [1]InteRNA Technologies, Utrecht, The Netherlands, [2]Utrecht Institute for Pharmaceutical Sciences, Utrecht University, The Netherlands, [3]Department of Urology, Radbout University Medical Center, Centre for Molecular Life Sciences, Nijmegen, The Netherlands, [4]Vrije Universiteit Amsterdam Medical Center, Amsterdam, The Netherlands, [5]Hubrecht Insitute, Cancer Genomics Center, University Medical Center, Utrecht, The Netherlands.

microRNA (miRNA) genes transcribed by RNA polymerase II generate small noncoding miRNAs of 18 to 24 nucleotides after maturation process. The mature miRNAs and their associated isomirs specifically bind to different mRNA transcripts, resulting in down regulation of multiple genes within the cell in a highly multiplexed way. miRNA expression profiles differ between human cell types suggesting cell-specific impacts of each miRNA on the regulation of different biological processes. Comparison of miRNA profiles of tumor samples and adjacent normal tissues showed that some miRNAs are up- or down-regulated and suggested their implication during tumor progression. However, such a miRNA profiling approach is not sufficient to identify the respective role of each miRNA gene during the tumorigenesis.

Here, to assess the individual role of each miRNA gene and its different isomirs in a specific cell environment, we have constructed a lentiviral miRNA expression library containing more than 1100 human known and novel miRNA precursors. The arrayed layout of our library allowed high-throughput screens with a large spectrum of functional read-outs using either normal or tumor cells. To exemplify this approach, the results of three different screens will be presented; i.e. identification of miRNAs that inhibit the BRAF pathway, miRNAs that inhibit tumor angiogenesis and miRNAs that stimulate the mesenchymal to epithelial transition. In addition, beyond this hit identification step, we will present detailed characterization of the role of the identified miRNAs in tumor progression by means of molecular and cellular functional assays.

Combining our unique miRNA expression library with a functional screening platform has allowed the identification and the further characterization of several miRNAs able to significantly impact on tumor behavior supporting the therapeutic interest of some candidates.

A13 The microRNA-200 family overcomes cisplatin resistance by targeting ceramide glycosylation. Chunxiao Cai[1], Hassan Ashktorab[2], Xiaowu Pang[2], Yuan Zhao[2], Wei Sha[2], Yulan Liu[1], Xinbin Gu[2]. [1]Peking University, Beijing, China, [2]Howard University, Washington, DC.

Background: Chromodomain-helicase-DNA-binding protein 5 (CHD5) is a newly identified tumor suppressor that is frequently downregulated in a variety of human cancers. Our previous work revealed that the low expression of CHD5 in colorectal cancer is correlated with CHD5 promoter CpG island hypermethylation. In this study, we investigated the effect of microRNA-211 (miR-211)-regulated CHD5 expression on colorectal tumorigenesis. Methodology/ Principal Findings: miR-211 was predicted to target CHD5 by TargetScan software analysis.

A stably expressing exogenous miR-211 colorectal cancer cell line (HCT-116miR-211) was generated using lentiviral transduction and used as a model for in vitro and in vivo studies. The expression level of miR-211 in HCT-116miR-211 cells was upregulated by 16-fold compared to vector control cells (HCT-116vector). Exogenous miR-211 directly binds to the 3' untranslated region (3' UTR) of CHD5 mRNA, resulting in a 50% decrease in CHD5 protein level in HCT-116miR-211 cells. The levels of cell proliferation, tumor growth, and cell migration of HCT-116miR-211 cells were significantly higher than HCT-116vector cells under both in vitro and in vivo conditions, as determined using the methods of MTT, colony formation, flow cytometry, tumor xenografts, and scratch assay, respectively. In addition, we found that enforced expression of miR-211 in HCT-116 cells was able to alter p53 pathway-associated regulatory proteins, such as MDM2, Bcl-2, Bcl-xL, and Bax. Conclusion/Significance: Our results demonstrate that CHD5 is a direct target of miR-211 regulation. Enforced expression of miR-211 promotes tumor cell growth at least in part by downregulating the expression level of the CHD5 tumor suppressor. Our results provide a better understanding of the association of between miR-211-regulated CHD5 expression and CHD5 function in colorectal tumorigenesis.

A14 microRNA-mediated interactions regulate established oncongenic pathways in cancer. Pavel Sumazin, Hua-Sheng Chiu, Andrea Califano. Columbia University, New York, NY.

By analyzing gene expression data in gliobastoma in combination with matched microRNA profiles, we have uncovered a post-transcriptional regulation layer of surprising magnitude, comprising hundreds of thousands of microRNA -mediated interactions. These include ~7,000 genes whose transcripts act as microRNA 'sponges' and 148 genes that act through alternative, non-sponge interactions. Biochemical analyses in cell lines confirmed that this network regulates established drivers of tumor initiation and subtype, including PTEN, PDGFRA, RB1, VEGFA, STAT3, and RUNX1, suggesting that these interactions mediate crosstalk between canonical oncogenic pathways. RNA silencing of 13 microRNA-mediated PTEN regulators, whose locus deletions are predictive of PTEN expression variability, was sufficient to downregulate PTEN in a 3' UTR-dependent manner and to increase tumor-cell growth rates. Thus, this microRNA-mediated network provides a mechanistic, experimentally validated rationale for the loss of PTEN expression in a large number of glioma samples with an intact PTEN locus. Recently, we extended this work to three other tumor types, showing that microRNA-mediated interactions propagate the effects of genetic alterations to regulate distal oncogenes and tumor suppressors in multiple cancers. Our results suggest that, although implemented by context-specific factors, hundreds of thousands of these interactions are context-independent.

A15 Somatic mutations of KEAP1 gene in common solid cancers. An Changhyeok, Yoo Namjin, Kim Hyung Ran, Lee Sug Hyung. Catholic University Medical College, Seoul, Republic of Korea.

Aims: KEAP1 inhibits NRF2-induced cytoprotection and is considered to be a candidate tumor suppressor. Somatic mutation of NRF2 has been analyzed in wide types of human cancer, whereas somatic mutation of KEAP1 has been reported only in lung and gall bladder cancers. The aim of our study was to address whether KEAP1 mutation is widespread in human cancers.

Methods: We analyzed 499 cancer tissues from lung, breast, colon, stomach, liver, larynx and prostate, and leukemias by single-strand conformation polymorphism.

Results: We detected somatic mutations of the KEAP1 in gastric (11.1%), hepatocellular (8.9%), colorectal (7.8%), lung (4.6%), breast (2.0%) and prostate (1.3%) carcinomas. Allelic losses of KEAP1 locus were identified in 42.9% of cancers with the KEAP1 mutation, but no NRF2 mutation was detected in these cancers. NRF2-activated cytoprotective proteins (NQO1 and GCLC) were expressed in all of the cancers with the KEAP1 mutation.

Conclusions: Our data show that that KEAP1

mutation occurs widely in solid cancers irrespective of histologic types. Biallelic inactivation of KEAP1 gene and increased cytoprotective proteins in the cancers suggest that KEAP1 mutations might protect cancer cells from oxidative insults and play a role in the development of solid cancers.

A16 miR-22 downregulation via epigenetic control in oral cancer cells. Li-Wha Wu. National Cheng Kung University, Tainan, Taiwan.

The deregulation of certain microRNAs has been associated with the progression and metastasis of various cancer types. DNA methylation in the CpG islands plays a crucial role in the down-regulation of tumor suppressor genes, including miRNAs. MiR-22 has been shown to be deregulated in several cancer types, including breast cancer and multiple myeloma. Using real-time RT-PCR analysis, we also found that miR22 was down-regulated in the majority of tested oral cancer cell lines and 70% of the clinical specimens when compared with their normal counterparts. Consistent with the notion that DNA methylation might play a role in the gene silencing, we did detect a putative CpG island (~ 1.2 kb in length) located upstream from the putative miR-22 transcription start site (+1) using two independent CpG island prediction softwares. Two oral cancer lines with low miR-22 expression were treated for 3-5 days with the indicated dose of 5'-azaC together with or without trichostatin A. Total RNA was isolated from the treated cells for real-time quantitative PCR analysis of miR-22 expression. The expression of miR-22 was significantly induced by either inhibitor. Combined treatment further enhanced the expression, suggesting the involvement of epigenetic control in the decreased expression of miR-22 in oral cancer cells. We first divided the putative CpG island into 5 regions and designed nested PCR primers for bisulfate sequencing PCR. We found that only regions 3 to 5 in the putative CpG island were subjected to be influenced by methylation in certain oral cancer lines. Since MeCP2 selectively binds to methylated CpG dinucleotides, chromatin immunoprecipition together with qPCR will be used to examine if MeCP2 would have increased binding to the identified methylated CpG sites. Second, quantitative methylation specific PCR (qMSP) will be used to measure the methylation levels of miR-22 genes in oral cancer tissues and their normal control using SYBR Green–based detection technology. Third, in vitro methylation and promoter-driven luciferase assay will be used to investigate whether DNA methylation plays a direct role in regulating miR-22 promoter activity and whether CpG island deletion affects the relative luciferase activity. Together, this study would implicate that the expression of miR-22 is controlled by epigenetic control in oral cancer cells. However, the exact nature of miR-22 in oral carcinogenesis remains to be characterized.

A17 miR-15, miR-16, and miR-21 are direct transcriptional targets of E2F1 that limit E2F-induced proliferation and apoptosis. Matan Ofir, Berta Ben-Shachar, Dalia Hacohen, Doron Ginsberg. Bar-Ilan University, Ramat Gan, Israel.

microRNAs (miRs) are small non-coding RNA molecules that have recently emerged as critical regulators of gene expression and are often deregulated in cancer. In particular, miRs encoded by the miR-15a, miR-16-1 cluster are frequently deleted in certain cancers and appear to act as tumor suppressors. Furthermore, miR-21 is over-expressed in a wide variety of human tumors and functions as an oncogene. Here, we provide evidence that miR-21, the miR-15a, miR-16-1 cluster and related miR-15b, miR-16-2 cluster comprise miRs regulated by E2F1, a pivotal transcription factor that can induce both proliferation and cell death. E2F1 is a critical downstream target of the tumor suppressor RB. The retinoblastoma pathway is often inactivated in human tumors resulting in deregulated E2F activity. We show that expression levels of the five mature miRs, miR-15a, miR-16-1, miR-15b, miR-16-2 and miR-21 are elevated upon activation of ectopic E2F1. Moreover, activation of endogenous E2Fs up-regulates expression of these miRs and endogenous E2F1 binds their respective promoters.

Importantly, we corroborate that miR-15a/b inhibits expression of cyclin E, a key direct

transcriptional target of E2F pivotal for the G1/S transition. Similarly, miR-21 inhibits the expression of the pro-apoptotic tumor suppressor gene maspin, another direct transcriptional target of E2F. Therefore, our data suggest the existence of feed forward loops that modulate E2F activity and consist of E2F1; E2F1-regulated miRs -miR-15 and miR-21; and E2F1-regulated genes-cyclin E and maspin. In support of this, ectopic expression of miR-15 inhibits G1/S transition and, conversely, inhibition of miR-15 expression enhances E2F1-induced G1/S transition and up-regulation of cyclin E levels. Also, inhibition of miR-21 expression enhances E2F1-induced up regulation of maspin, as well as E2F1-mediated apoptosis.

In summary, our data identify miR-15, miR-16 and miR-21 as novel transcriptional targets of E2F that, in turn, modulate E2F activity.

A18 p53 regulates epithelial-mesenchymal transition through microRNAs targeting ZEB1 and ZEB2. Taewan Kim[1], Tae Jin Lee[1], Young-Jun Jeon[1], Pascal Pineau[2], Anne Dejean[2], Carlo Maria Croce[1]. [1]Ohio State University, Columbus, OH, [2]Institut Pasteur, Paris, France.

p53 suppresses tumor progression and metastasis. Epithelial-mesenchymal transition (EMT) is a key process in tumor progression and metastasis. The transcription factors ZEB1 and ZEB2 promote EMT. Here we show that p53 suppresses EMT by repressing expression of ZEB1 and ZEB2. By profiling 92 primary hepatocellular carcinomas (HCCs) and 9 HCC cell lines, we found that p53 upregulates microRNAs including miR-200 and miR-192 family members. The miR-200 family members transactivated by p53 then repress ZEB1/2 expression. p53-regulated miR-192 family members also repress ZEB2 expression. Inhibition or over-expression of the microRNAs affects p53-regulated EMT by altering ZEB1 and ZEB2 expression. Our findings indicate that p53 can regulate EMT, and that p53-regulated microRNAs are critical mediators of p53-regulated EMT.

A19 Profiling long noncoding RNA expression in cancer cells by real-time PCR arrays. Fangting Wu[1], Yin-Yuan Mo[2]. [1]System Biosciences, Mountain View, CA, [2]Southern Illinois University, Springfield, IL.

Long noncoding RNAs (lncRNAs), as a new class of transcripts, have been recently shown to be pervasively transcribed in the genomes of human and mouse. In particular, accumulating evidence link the expression of lncRNAs to diverse human diseases, highlighting their potential as biomarkers and therapeutic targets. However, there are still many missing dots in our current understanding of lncRNA function. Thus, investigation of the expression patterns of lncRNAs is a crucial step to understanding of their roles in many model systems. To this end, we developed a qPCR based disease-related human lncRNA profiler, which allows for the quantification of differential expression of 83 individual lncRNAs among various experimental RNA samples. All 83 lncRNAs chosen for the array are based on publications and they are implicated in diseases ranging from neurodegeneration to cancer. The array plate also includes the house keeping genes and small RNA transcripts for normalization purposes. To determine the utility of this lncRNA profiler, we profiled their expression in breast cancer specimens compared to matched normal tissue and identified several lncRNAs are dysregulated, suggesting that lncRNAs may function as oncogenes or tumor suppressors. Gene expression profiling with this lncRNA profiler in a series of breast cell lines such as MCF10A, MCF7 and MDA-MB-231 cells identified differential expression patterns unique to each cell line, which may provide a basis for future analysis of these differentially expressed lncRNAs. Furthermore, we determined the effect of p53 on lncRNA expression in HCT-116 and MCF-7 cells. Initial testing indicated that at least three lncRNAs are induced by p53 in both cell lines. Experiments are underway to further characterize these p53 inducible lncRNAs. Together, these results suggest that disease-related human lncRNA profiler is a valuable research tool that can be tailored for specific needs in different research laboratories. The

features of this profiler are easy, convenient, sensitive and specific. Accordingly it serves the first step toward the understanding of the role of lncRNAs in human diseases.

A20 The Six1-regulated miR-106b-25 cluster is a mediator of the tumor promotional effects of TGF-β signaling in human breast cancer. Anna L. Smith, Ritsuko Iwanaga, David J. Drasin, Douglas S. Micalizzi, Rebecca L. Vartuli, Heide L. Ford. University of Colorado Anshutz Medical Campus, Aurora, CO.

Recent studies have highlighted the developmental transcription factor Six1 as an important mediator of breast cancer progression and metastasis. Six1 is overexpressed in a striking 90% of metastatic breast cancer lesions, and patients whose tumors overexpress Six1 have a decreased time to metastasis and relapse, as well as an overall decrease in survival. Six1 mediates metastasis via multiple mechanisms, including its ability to induce an epithelial-to-mesenchymal transition (EMT) and tumor initiating cell (TIC) characteristics, both of which are dependent on an upregulation of TGF-β signaling. Indeed, the Six1-induced increase in TGF-β signaling is critical for the ability of Six1 to induce late stage metastasis. Interestingly, Six1 not only activates TGF-β signaling, but it also enables the switch of TGF-β signaling from tumor suppressive to tumor promotional, a phenomenon of considerable importance in cancer pathogenesis. This event, coined the "TGF-β paradox," has been an area of extensive research, but remains largely elusive.

To further investigate the mechanism by which Six1 mediates the switch in TGF-β signaling, we performed a miRNA microarray screen and identified a cluster of miRNAs, the miR-106b-25 cluster, that is upregulated in response to Six1 overexpression. The miR-106b-25 cluster consists of three miRNAs, miR-106b, miR-93, and miR-25, which reside together in the intron of the MCM7 gene. Importantly, overexpression and knockdown experiments demonstrate that Six1 regulates all three miRNAs within the cluster. Interestingly, these miRNA have previously been implicated in the impairment of TGF-β-mediated growth suppression through repression of the cell cycle inhibitor, p21, and pro-apoptotic factor, Bim. These data suggest that Six1-induced upregulation of these miRNA may mediate the switch in TGF-β signaling from tumor suppressive to tumor promotional. Surprinsingly, bioinformatic analysis revealed that the miR-106b-25 cluster may also contribute to the activation of TGFβ signaling through repression of the TGF-β signaling inhibitor, Smad7, which mediates the degradation of TβRI. Indeed, overexpression of the miR-106b-25 cluster results in repression of the Smad7 protein, with concominant upregulation of TβRI. Furthermore, activation of TGF-β signaling is observed with miR-106b-25 overexpression, as demonstrated by an increase in phosphorylation of the downstream effector of the TGF-β pathway, Smad3, and by an upregulation of downstream TGF-β transcriptional targets. miRNA inhibition also demonstrates that miR-106b and miR-93 are necessary for Six1 induced TGF-β activation. Additionally, like Six1, the miR-106b-25 cluster is sufficient to induce features of EMT, including a redistribution of E-cadherin, and increased β-catenin transcriptional activation. Furthermore, the miR-106b-25 cluster is also sufficient to increase the TIC population as measured by flow cytometry, mammosphere formation, and in vivo serial dilution assays. Finally, we demonstrate a significant correlation between miR-106b, Six1, and activated TGF-β signaling in human breast cancers, and further show that high levels of miR-106b and miR-93 in breast tumors significantly predicts shortened time to relapse. These findings expand the spectrum of oncogenic functions of miR-106b-25, and may provide a novel molecular explanation, through the Six1 regulated miR-106b-25 cluster, by which TGF-β signaling shifts from tumor suppressive to tumor promotional.

A21 MALAT-1 is essential for lung cancer metastasis in a novel human knockout model. Tony Gutschner[1], Moritz Eißmann[2], Monika Hämmerle[1], Marion Stentrup[1], Catherina Hildenbrandt[1], Matthias Groß[1], Martin Zörnig[2], Sven Diederichs[1]. [1]German Cancer Research Center (DKFZ) & Institute of Pathology, Heidelberg, Germany, [2]Georg-Speyer-Haus, Frankfurt, Germany.

The highly conserved long non-coding RNA MALAT-1 (Metastasis-Associated in Lung Adenocarcinoma Transcript 1) had been discovered as a prognostic marker associated with poor survival and development of distant metastasis in lung adenocarcinoma. Since then, it has been found to be deregulated in numerous tumor entities and has been linked to splicing. However, its functional relevance in tumor cells remains to be elucidated. Knockdown models for MALAT1 have been described but suffer from insufficient silencing efficiency of the highly abundant, nuclear non-coding RNA (ncRNA).

In this project, we have developed a novel strategy to create ncRNA knockouts in human cancer cell lines. We have successfully used a synthetic Zinc Finger Nuclease engineered to target the 5'-region of MALAT1 to stably and biallelically integrate RNA-destabilizing elements into the genome of human lung cancer cells (A549). This approach resulted in a specific and more than 1000-fold silencing of MALAT1 in individual clones compared to a less than 5-fold silencing using siRNAs. Thus, this approach can be used to create functional knockouts of coding as well as non-coding genes also in human tumor cell lines allowing loss-of-function studies also of non-conserved ncRNAs in the future.

Phenotypically, the MALAT1-Knockout cells (KO) greatly differ from their parental cell line and wildtype clones (WT): Next to morphological changes, the migration of the KO cells is largely impaired as shown in scratch assays. In xenograft assays after i.v. injection, the KO cells form significantly fewer and smaller lung metastases than their WT counterparts. Since no large difference was observed after subcutaneous injection of the WT and the KO cells, this indicates a specific, active and essential function of MALAT1 in metastasis.

Exon microarrays of the WT and KO cell lines have revealed multiple migration- or metastasis-associated transcripts deregulated by loss of MALAT1 and hence potential target genes. These analyses also uncovered, that splicing is likely not the only functional mechanism of MALAT1 in the nucleus.

Taken together, we have developed a novel, highly effective approach for the knockout of genes that can be used for non-coding as well as coding RNAs in human tumor cells as well as cells from other species. Knockout of MALAT1 in human lung cancer cells revealed its essential function in metastasis as well as genes directly and indirectly targeted by MALAT1.

A22 Genome-wide miRNA screens revealed miRNAs that regulate MGMT expression. Deepa Kushwaha[1], Valya Ramakrishnan[2], Bob Carter[2], Clark C. Chen[2]. [1]Dana-Farber Cancer Institute, Boston, MA, [2]Moores Cancer Center, University of California, San Diego, CA.

DNA alkylating agents remain the critical chemotherapeutic agents for a wide spectrum of cancers, including glioblastoma. The tumoricidal activity of DNA alkylating agents is largely derived from the induction of O^6-alkyl-guanine which is the major carcinogenic lesion in DNA. A major mechanism of therapeutic resistance involves the DNA repair enzyme O^6- methylguanine-DNA methyltransferase (MGMT). The primary role of this enzyme is to remove the alkyl group from the modified guanine and restore the DNA to its undamaged form. This enzyme plays a critical role in modulating the therapeutic efficiency of DNA alkylating chemotherapies, including temozolomide, in the treatment of glioblastomas. In glioblastoma, low levels of MGMT expression are associated with favorable response to temozolomide therapy.

In an analysis of tumor specimens derived from glioblastoma patients, we found significant disparity in the expression levels of MGMT mRNA and protein in a large number of specimens. The MGMT mRNA levels correlated poorly with MGMT protein levels by Western blotting (R^2=0.04, p=0.34)

or by ImmunoHistoChemical (IHC) stain quantitation (R^2=0.02, p=0.50). To exclude the possibility that the poor correlation was due to substandard specimens, we determined the mRNA and protein levels of Colony Stimulating Factor 1 (CSF1), a gene previously shown to exhibit excellent mRNA/protein correlation. In contrast to MGMT, the mRNA expression of CSF1 correlated well with protein (R^2=0.47, p=0.001).

We found that a major contributor to this disparity involved the expression of MGMT regulating microRNA. We hypothesized that the increased expression of such microRNAs would be associated with favorable response to temozolomide and looked for such miRNAs by a genome-wide microRNA profiling screen using specimens derived from 87 glioblastoma patients and correlating these findings with temozolomide response. We identified miR-181d as one such candidate. Indeed, transfection of miR-181d down-regulated MGMT mRNA and protein expression. Further, luciferase reporter assays and co-precipitation studies revealed direct interactions between miR-181d and the MGMT 3' UnTranslated Region (UTR). Finally, MGMT expression inversely correlated with miR-181d expression in independent glioblastoma collections. However, this correlation was modest.

The modest correlation between miR-181d and MGMT suggests the existence of other MGMT regulating miRNAs. We thus carried out a biochemical screen transfecting 885 known miRNAs into MGMT expressing T98 glioblastoma cells to identify miRNAs that down-regulated MGMT expression upon transfection. The top 15 candidates identified were then tested in A1207, LN18 and LN340 glioblastoma cells, three independent MGMT expressing cell lines. We found that miR-603 consistently down-regulated MGMT expression on both the protein and mRNA level. The effect was observed in both conventional and neurosphere glioblastoma lines. Importantly, the suppression of MGMT expression and induction of temozolomide sensitivity by miR-603 could be reversed by the expression of miR-603 antagomirs. Further, the luciferase activity from the construct containing the MGMT 3'UTR was reduced after transfection of miR-603 relative to a control miRNA. Mutating the putative miRNA binding sites abolished these effects. Additionally, MGMT mRNA preferentially immunoprecipitated with biotinylated miR-603. Finally, we demonstrated that the high expression of miR-603 correlated with improved clinical response to temozolomide in two independent clinical datasets.

Overall, our data suggest that MGMT expression is regulated by multiple miRNAs. Further, the expression level of such miRNAs correlate with clinical response to temozolomide treatment. An exhaustive identification of such miRNAs may harbor implications in terms of personalizing oncologic care as it relates to alkylating chemotherapies.

A23 A noncoding RNA panel predicts intermediate-risk childhood rhabdomyosarcoma prognosis better than standard pathologic criteria and coding genes. Anirban P. Mitra[1], Jonathan D. Buckley[1], Sheetal A. Mitra[2], Elai Davicioni[3], James R. Anderson[4], Philipp Kapranov[5], Stephen X. Skapek[6], Douglas S. Hawkins[7], Timothy J. Triche[2]. [1]University of Southern California, Los Angeles, CA, [2]Children's Hospital Los Angeles, Los Angeles, CA, [3]GenomeDx Biosciences Inc., Vancouver, BC, Canada, [4]University of Nebraska, Omaha, NE, [5]The St. Laurent Institute, Cambridge, MA, [6]UT Southwestern Medical Center, Dallas, TX, [7]Seattle Children's Hospital, Seattle, WA.

Introduction: Rhabdomyosarcoma (RMS) is the most common pediatric soft-tissue sarcoma, stratified by the Children's Oncology Group (COG) into low/intermediate/high risk based on clinical outcomes. However, most patients are categorized as intermediate-risk where survival is highly heterogeneous, thus suggesting an inability to accurately stratify a majority of patients. We profiled intermediate-risk RMS's for levels of coding and non-coding transcripts to construct prognostic signatures. The goal was to identify panels of RNAs that reflect underlying tumor biology and provide better risk stratification than routine clinicopathologic parameters.

Methods: Transcriptomes from 79 prospectively-obtained primary tumors from intermediate-risk RMS patients under COG clinical trial protocols were profiled on Affymetrix Human Exon 1.0 ST microarrays. Expressions of 1,400,033 probe sets representing annotated and unannotated transcripts were analyzed using Genetrix suite of microarray analysis tools. Cox regression and leave-n-out cross validation were used to derive and finalize the expression signatures. An effort was made compare individual prognostic potentials of the coding and non-coding signatures, and that of a signature that combined both features.

Results: Standard pathologic prognosticators such as histologic subtype classification (alveolar versus embryonal) and *PAX-FKHR* fusion gene status were unable to predict outcome in this cohort (p=0.40 and 0.45, respectively). Cox regression analysis on 17,049 coding transcripts created a 42-gene meta-feature that was able to predict survival (p=0.00024). Leave-n-out cross validation of this meta-feature upheld its prognostic ability (p=0.00030). Analysis of probe set regions (PSRs) corresponding to unannotated "dark matter" transcripts identified a 32-PSR meta-feature that also predicted survival with greater significance than PSRs corresponding to coding transcripts (p<0.00001). To reduce feature redundancy, multiple PSRs interrogating the same genomic locus were replaced by a representative PSR that shrunk the meta-feature size to 24 PSRs, which was still able to predict survival better than the coding gene meta-feature (p<0.00001). A meta-feature that combined coding and non-coding RNA features retained its ability to predict outcome (p=0.00002), with non-coding RNA features contributing towards the bulk of its prognostic potential.

Conclusions: A more concise non-coding RNA meta-feature was able to better predict outcome than a larger coding gene meta-feature in intermediate-risk RMS, where standard pathologic prognosticators failed. This suggests the role of non-coding transcripts in regulating and determining RMS biology and aggressiveness, and their potential to serve as novel prognostic indicators.

A24 Interaction of miR-106a~363Xpcl1 and p27Kip1 in thymopoiesis and lymphomagenesis. Daniel Kuppers, Thomas Schmitt, Bruce Clurman, Harry Hwang, Matthew L. Fero. Fred Hutchinson Cancer Research Center, Seattle, WA.

The *Xpcl1 (Kis2)* gene encodes the miR-106a~363 miRNA cluster in mice. Although it was first identified as common integration site of the M-MuLV retrovirus in tumors from $p27^{Kip1}$ *(Cdkn1b)* knockout mice, the nature of its interaction with cell cycle genes is uncertain. The miR-106a~363 miRNAs are highly conserved across species, implying an important role in normal biological processes. However, in the absence of a reported phenotype in knockout mice, its normal function is unclear. We examined miR-106a~363 expression in developing T lymphocytes and found that it is highly expressed in immature T-cells but is repressed at the CD4+/CD8+ double positive (DP) stage of thymopoiesis. Using a T cell specific Xpcl1 transgene, we found that forced expression of miR-106a~363 altered the distribution of T cell subsets. *Xpcl1* repressed T-cell receptor expression and directly targeted the CD69 cell surface protein. This causes accumulation of DP thymocytes through complementary mechanisms.

We show that, in developing T-cells, $p27^{Kip1}$ is differentially regulated due to variation in expression of FoxO family transcription factors. Likewise, p27 is upregulated at the transcriptional level by Xpcl1, which is surprising since this should have a tumor suppressor effect. Nonetheless, miR-106a~363 transgenic (Lx) mice developed spontaneous high grade T cell lymphomas. The combination of $p27^{Kip1}$ deletion with miR-106a~363 overexpression, markedly accelerated lymphoma development, confirming cooperation of these two mutations. We conclude that deletion of $p27^{Kip1}$ directly overcomes a tumor-suppressor activity of miR-106a~363, whereas the principal oncogenic function of the miRNA cluster remains unknown.

A25 Differences in microRNA expression patterns in breast cancer subtypes. Sandra L. Romero-Cordoba[1], Rosa G. Rebollar-Vega[1], Valeria Quintanar-Jurado[1], Veronica Bautista-Pina[2], Sergio Rodriguez-Cuevas[2], Antonio Maffuz-Aziz[2], Alfredo Hidalgo-Miranda[1]. [1]Instituto Nacional de Medicina Genomica, Mexico, [2]Instituto de Enfermedades de la mama FUCAM, Mexico.

Breast cancer can be classified according to the gene expression signatures of the tumors into four clinically relevant sub-types, luminal A, luminal B, Her2-like and basal-like. Additionally microRNA expression patterns can separate normal tissue from breast tumors, but there is currently limited information about the potential differences in microRNA expression profiles between the different tumor subtypes, defined by gene expression. In order to determine the microRNA expression profiles in breast tumors, and explore the differences in the expression patterns of these molecules between different cancer subtypes, we analyzed the expression of 664 microRNAs with the TaqMan low-density array platform in 40 breast tumors from different subtypes, and in 21 adjacent normal breast tissues. Tumor sub-typing was carried out with the PAM50 algorithm in expression data obtained with the Affymetrix Human Gene ST 1.0 array, obtaining 8 Luminal A tumors, 9 luminal B, 8 Her2-like and 3 Triple negative basal-like tumors. Given the low number of basal-like tumors, for the microRNA expression analysis, we included 12 additional triple-negative tumors defined by immunohistochemistry. Finally, we analyzed the expression of DICER and Ago2, involved in the biogenesis of microRNAs in the breast tumors. 131 microRNAs showed significant differential expression (adjusted P value=0.05, Fold Change=2) in breast tumors compared to the normal adjacent tissue. The role of 25% of these microRNAs has not been previously reported in breast cancer. 10 microRNAs showed differential expression between basal/triple negative tumors compared to hormone receptor and HER2 positive tumors. Transcriptional targets of these microRNAs include genes involved in the carcinogenesis of triple negative tumors, like PARP1, or in the microRNA biogenesis machinery, like DICER. Enrichment ontology analysis of the microRNAs differentially expressed in the TN tumors, detected pathways like p53 and focal adhesion whose role in cancer development, invasion and metastasis might be crucial; and MAPK, which has been related to recurrence of TN tumors. Regarding the expression of Ago2 and DICER, we detected down-regulation (approximately 20% less) of both proteins, mainly in TN tumors. Down-regulation in the triple negative tumors was further validated at the mRNA level by RT-qPCR assays. Our data identified the altered expression of several microRNAs whose aberrant expression might have an important impact on cancer-related cellular pathways and whose role in breast cancer has not been previously described. microRNAs expression is also capable to discriminate between different tumor subtypes and the expression of proteins involved in microRNA biogenesis might play a role in breast carcinogenesis, specifically in the triple negative subtype.

A26 DNA copy number alterations deregulate expression of miRNAs in lung adenocarcinoma. Greg L. Stewart, Katey SS Enfield, Stephen Lam, Wan Lam. British Columbia Cancer Research Centre, Vancouver, BC, Canada.

Background: Lung cancer represents an enormous health burden, representing the most common cause of cancer death worldwide, with a five-year survival of less than 15%. microRNAs (miRNAs) have emerged as major players in lung cancer oncogenesis, displaying both oncogenic and tumor suppressive functions. DNA copy number (CN) amplification of oncogenes is a major molecular mechanism driving cancer, and like protein coding genes, CN alterations can influence miRNA expression levels. We hypothesize that DNA copy number gain modulates the expression of miRNAs important to cancer cell growth, and that integrative analysis can identify these cancer driving miRNAs.

Methods: Global gene dosage profiles for 46 lung adenocarcinoma and paired adjacent non-malignant tissues were generated by array comparative genomic hybridization. miRNA sequencing analysis was performed

on this same panel of tumors and matched non-malignant tissues using Illumina GAXII small RNA sequencing technologies. CN and expression were correlated for each miRNA (Spearman's correlation >0.3, p<0.05) and expression of each significant miRNA was compared between tumors with and without CN gain (U-test p<0.05). To select for miRNAs that most likely play a role in cancer we compared the expression between tumor and matched normal and selected for miRNAs with a fold change of at least 2 in tumors as compared to normal tissues.

Results: We identified a panel of miRNAs in lung adenocarcinoma whose expression deregulation was associated with CN alteration. These miRNAs were found to be gained in >15% of tumors and underwent at least a 2 fold greater increase in expression in tumors compared to matched normal tissues. This study identified several miRNAs known to play a role in cancer including miR-141, known to be highly upregulated in lung and ovarian cancer, and miR-301a, upregulated in invasive early cervical cancer. In addition we identified several novel miRNAs that have not yet been implicated in cancer.

Conclusion: Here we identify a panel of miRNAs that are selectively gained at the genomic level to alter expression in lung adenocarcinoma. Several of these miRNAs have been previously shown to have oncogenic properties in many cancer types including lung cancer and may be potentially useful as therapeutic targets. In addition we identify several miRNAs that have not been previously reported in lung cancer. Future characterization of these miRNAs may lead to increased knowledge of adenocarcinoma oncogenesis.

A27 microRNA 21 in adipocyte-derived microvesicles confers Taxol-resistance of ovarian cancer cells through downregulation of apoptotic protease activating factor 1. Ngai Na Co, Rosemarie Schmandt, Karen Lu, Samuel Mok. The University of Texas MD Anderson Cancer Center, Houston, TX.

Over the past 30 years, there has been a dramatic increase in the incidence of obesity worldwide. As of 2010, approximately one-third of U.S. adults are obese, while another one-third of adults are overweight. Obesity is one of the risk factors for ovarian cancer. Greater than 75% of ovarian cancer patients present with advanced stage disease. Tumors preferentially spread to the omentum, a major site of abdominal fat, where ovarian cancer cells infiltrate omental adipose tissue. Omental adipocytes may interact directly with cancer cells to create a microenvironment niche that supports cancer growth. Recent studies have shown that cells can communicate through the exchange of bioactive molecules via microvesicles (exosomes and ectosomes). Microvesicles fuse with target cell membranes and transfer proteins, lipids, mRNAs and non-coding RNAs from donor to recipient cells. We hypothesize that adipocytes secrete microvesicles containing tumor-supporting factors that promote ovarian cancer progression.

Ion Torrent RNA sequencing and subsequent qRT-PCR analyses were used to identify non-coding microvesicle RNAs secreted by primary cultures of cancer associated adipocytes (CAA), cancer associated fibroblasts (CAF), normal adipocytes, normal fibroblasts and ovarian cancer cells. Of the non-coding microvesicle RNAs identified, microRNA 21 (miR21) showed significantly higher expression in microvesicles secreted from CAA when compared to those from CAF and normal omental adipocytes, fibroblasts, and ovarian cancer cells. Using qRT-PCR of RNA isolated from microdissected frozen tissue and In-situ hybridization on FFPE tissue sections, CAA demonstrated significant higher miR21 expression levels compared to the adjacent cancer cells and normal adipocytes. MiR21 was up-regulated in metastatic ovarian cancer cells in the omentum as compared to those in the primary tumor site. Moreover, strong miR21 staining was observed in omental cancer cells particularly at the invasion front, suggesting that miR21 may be transferred from CAA to their neighboring ovarian cancer cells.

To test this hypothesis, SKOV3 ovarian cancer cells were co-cultured with adipocytes transfected with miR21-FAM or incubated with fluorescently labeled adipocyte-derived

microvesicles. Confocal microscopy confirmed the delivery of miR21 by adipocyte-derived microvesicles to cancer cells. Since miR21 expression in tumor cells has been linked with resistance to a variety of chemotherapeutic agents, we further delineated the direct effect of miR21 on the chemoresistance of ovarian cancer cell. The ovarian cancer cell lines, OVCA432 and SKOV3, transfected with miR21 precursor showed an increase in cell survival and a decrease in apoptosis in the presence of taxol. Furthermore, miR21 was significantly up-regulated in taxol-resistant HeyA8-MDR and SKOV3-TR cells as compared to the parental cell lines, HeyA8 and SKOV3, respectively. These data strongly suggest that miR21 may confer taxol resistance to ovarian cancer cells. Using microarray analysis of RNA isolated from SKOV3 cells transfected with the miR21 precursor, we identified a set of chemoresistance-related genes associated with miR21 expression. One of the most significantly down-regulated genes is Apoptotic Protease Activating Factor 1 (APAF1). By qRT-PCR, we confirmed that miR21 over-expression decreased APAF1 mRNA expression levels in OVCA432 and SKOV3 cells. A strong miR21 binding site is predicted on the 3'-UTR of APAF1, suggesting APAF1 is the direct target of miR21.

In summary, up-regulation of miR21 in omental ovarian cancer cells is mediated through transport of miR21 in adipocyte-derived microvesicles from CAA in the omentum. Further, miR21 confers taxol-resistance in ovarian cancer cells by down-regulating APAF1 expression in ovarian cancer cells to decrease taxol-induced apoptosis.

A28 DD3/PCA3 noncoding RNA regulates prostate cancer cell survival and modulates AR signaling. Luciana Ferreira[1], Antonio Palumbo[2], Kivvi Mello[1], Felipe Leite[2], Cinthya Sternberg[1], Mauricio Caetano[1], Adriana Neves[3], Luiz Ricardo Goulart[3], Etel Gimba[4]. [1]Instituto Nacional de Câncer, Rio De Janeiro, Brazil, [2]Universidade Federal do Rio de Janeiro, Rio De Janeiro, Brazil, [3]Universidade Federal de Uberlândia, Uberlândia, Brazil, [4]Universidade Federal Fluminense, Rio Das Ostras, Brazil.

The prostate cancer antigen 3 (DD3/PCA3) is a non-coding RNA (ncRNA) specifically expressed in prostate tissues and overexpressed in prostate cancer (PCa) tumors. Although widely applied as a diagnostic marker for PCa, to date nothing has described about its role in PCa biology. We used herein small interfering RNA (siRNA) in order to knockdown DD3 mRNA message as an approach to elucidate DD3 functional roles in PCa cells. LNCaP cell line was used herein as an in vitro model for DD3 functional assays. siRNA sequences were specifically designed for DD3 exon 4 mRNA sequences (siDD3), as well an scrambled siRNA (siScr), as negative control. LNCaP cells were transiently transfected with siDD3 or siScr and DD3 expression was analysed by real time PCR (qRT-PCR) using DD3 specific oligonucleotides. LNCaP cells transfected with siDD3 demonstrated a marked decrease in cell proliferation and viability, as compared to siScr transfected cells. This growth inhibition was specific for DD3 expressing cells. Further, LNCaP cells in which DD3 was knocked-down presented a significant increase in proportion of cells in SubG0/G1 phase of cell cycle and presenting pyknotic nuclei, indicative of cells undergoing apoptosis. In order to investigate the putative mechanisms underlying the decrease of LNCaP cell survival as a result of DD3 knockdown, we then evaluated the involvement of DD3 on androgen receptor (AR) pro-survival signaling. DD3 expression was significantly upregulated as a result of LNCaP treatment with dihydrotestosterone (DHT), the active androgen metabolite. This effect was reverted by the addition of the AR antagonist, flutamide. Consistent to an AR activation by DHT treatment, LNCaP cells presented a significant upregulation

of AR target genes. Notably, siDD3/LNCaP transfected cells significantly inhibited the expression of tested AR responsive genes. Besides, DD3 knockdown was able to counteract DHT stimulatory effects over AR target gene expression. Despite negatively modulating the transcription of AR target genes, DD3 knockdown did not alter Akt and ERK phosphorylation, suggesting that DD3 is mainly controlling the expression of signaling pathways downstream to AR activation. Besides, DD3 mRNA expression was mainly detected in nuclei and ribosome cell compartments, indicating that in this cell niches DD3 ncRNA exert its main functional roles. In summary, our findings indicate that DD3 is a ncRNA whose expression is AR regulated and is involved on the control of PCa cell survival and proliferation, in part by modulating the AR signaling pathway and its target genes. These findings correspond to the first description of DD3 roles on PCa cells and could provide new insights into understanding prostate carcinogenesis, besides opening new prospects to use DD3 not only as a biomarker for PCa, but also as an specific target for therapeutic approaches aiming to inhibit PCa growth through negatively modulating AR pro-survival signal and their target genes.

A29 Notch-dependent modulation of microRNAs in rhabdomyosarcoma. Rossella Rota, Sr.[1], Roberta Ciarapica, Jr.[1], Letizia Da Sacco, Jr.[1], Maria De Salvo, Jr.[1], Andrea Masotti, Sr.[1], Lucio Miele, Sr.[2], Franco Locatelli, Sr.[1]. [1]Ospedale Pediatrico Bambino Gesu', Rome, Italy, [2]University of Mississippi Medical Center, Jackson, MS.

Rhabdomyosarcoma (RMS) is a pediatric tumor that originates from skeletal muscle precursors. Therapeutic strategies aimed at restoring the differentiation potential of RMS tumor cells are accepted as anti-cancer approaches. Therefore, pathways involved in skeletal muscle determination are good potential targets. We have recently demonstrated that Notch3 signaling is over-activated in RMS cell lines of both embryonal and alveolar subtype. Down-regulation of Notch3 expression resulted in cell cycle inhibition of RMS alveolar and embryonal cell lines that showed the appearance of multinucleated structures indicative of skeletal muscle-like myotubes in vitro. Moreover, Notch3 knockdown impaired tumor growth in vivo. Here we expand this study evaluating, through microarrays profiling, the expression of microRNAs 48 hours after Notch3 siRNA treatment compared to that of control siRNA. Several up- or down-regulated microRNAs were detected in both RH30 alveolar and RD embryonal cells. The up-regulation of muscle-specific microRNAs such as miR-1, miR-133 and miR-206 was confirmed 24 and 48 hours post-Notch3 depletion by RT-qPCR. Moreover, non-muscle-specific microRNAs such as miR-26a and miR-214 that cooperate to myogenesis were up-regulated as well. Altogether, these preliminary results indicate that Notch3 prevents the differentiation of RMS cells also through down-regulation of pro-myogenic microRNAs. More deep studies are in course to dissect the Notch3-dependent modulation of these small non-coding RNAs in this soft tissue sarcoma.

A30 miRNA regulators of ovarian cancer metastasis that predict patient outcomes. Alexander S. Brodsky[1], Hsin-Ta Wu[1], Souriya Vang[1], Benjamin Raphael[1], Laurent Brard[2]. [1]Brown University, Providence, RI, [2]Southern Illinois University School of Medicine, Springfield, IL.

Serous ovarian cancer patients often succumb to aggressive metastatic disease, yet little is known about the behavior and genetics of ovarian cancer metastases. We selected 12 matched primary and omental metastatic serous epithelial ovarian tumors to identify the copy number, mRNA and miRNA expression differences. No significant recurring copy number changes were detected. In contrast, significant recurrence with the same expression changes in all or the large majority of patients was observed for many mRNAs and miRNAs. We identify almost 1,000 genes with recurring significant expression differences across the patient cohort suggesting common phenotypic features are selected from heterogeneous mixture of primary tumors. Genes differentially expressed between metastatic and primary tumors identify a

poor prognosis subtype in primary tumors that robustly distinguishes high risk patients across multiple platforms in two large independent data sets. Multivariate analysis suggests that the expression signature is independent of residual disease, a common clinical risk factor. To gain insight into the regulation of these mRNAs, we measured the expression of 377 miRNAs using Taqman qPCR in matched primary and metastatic serous ovarian tumors. We identified 17 miRNAs with significant differential expression in primary and metastatic ovarian human tumors including miR-21, miR-31, and novel miRNAs previously not associated with metastasis. Many of these miRNAs have >10 fold expression differences across multiple patients. We confirmed that the expression differences originate from cancer cells for many of the miRNAs by in situ hybridization and laser capture microdissection of cancer cells from tumors followed by qPCR. We identify combinations of metastatic miRNAs with significantly stronger predictions of patient outcomes than random combinations of miRNAs in The Cancer Genome Atlas data. Some of these same miRNAs show the same expression changes in liver metastases compared to colorectal primary tumors. Ovarian cancer cells form multicellular aggregates, or spheroids, as they disseminate throughout the peritoneal cavity and we find that these metastatic miRNAs affect spheroid formation and growth. All 7 of the metastatic miRNAs expressed in two ovarian cancer cell lines are up-regulated in spheroids compared to monolayers, recapitulating the observations in human metastases compared to primary tumors, suggesting that similar adaptations required for 3D culture are needed to establish metastases. Interestingly, we find miR-31 promotes metastasis in ovarian cancer suggesting a context dependent function compared to breast cancer. miR-31 is up-regulated in metastases by qPCR and in situ hybridization, up-regulated in spheroids compared to monolayers and inhibition reduces spheroid size and viability using three different inhibitors without significant effects on monolayer growth. We have tested the function of 6 other metastatic miRNAs, which predict patient survival, using both gain and loss of function experiments. We find that many of the metastatic miRNAs mediate colony formation, mobility, and/or spheroid size, but do not significantly affect monolayer culture. Predicted targets negatively correlate with miRNA expression better than sets of random permutations in the tumors, and some of these targets negatively correlate with miRNAs in spheroids vs. monolayers. These metastatic miRNAs appear to promote metastasis in part by enhancing β-catenin signaling through repression of APC and suppressing apoptosis. Using miRNA expression profiles and functional studies, we have established the utility of spheroid cultures to examine these clinically relevant metastatic miRNAs. In sum, we have identified metastatic miRNAs, from one of the first miRNA profiles of metastases, critical for aggressive disease in ovarian, and perhaps other, cancers, with potential for biomarker and therapeutic development. We find that multiple miRNAs, many expressed in metastases and not in primary tumors in some patients, are likely important to drive metastasis by regulating multiple gene networks.

A31 Differential processing of let-7a precursors influences gemcitabine chemosensitivity in pancreatic cancer: Role of ribonucleotide reductase subunit M2. Rajgopal Govindarajan. University of Georgia, Athens, GA.

Ribonucleotide reductase subunit M2 (RRM2) involved in deoxyribonucleotide synthesis acts as a key determinant of chemoresistance to nucleoside drugs (e.g., gemcitabine) used in treating pancreatic cancer. While silencing RRM2 expression by synthetic means (e.g., siRNA) improves the chemosensitivity of pancreatic cancer cells to gemcitabine, targeting endogenous molecules to advance gemcitabine chemotherapeutic responses has never been explored. Based on existing evidence and computational predictions, we hypothesized that the let-7 tumor suppressor microRNAs will inhibit RRM2-mediated gemcitabine chemoresistance in pancreatic cancer. Reduced expression of the majority of let-7 miRNAs with an inverse relationship to RRM2 expression was identified in inherently gemcitabine-resistant pancreatic

cancer cells. Direct binding of let-7 miRNA to the 3'-UTR of RRM2 transcripts identified post-transcriptional repression of RRM2 as a novel phenomenon influencing gemcitabine chemosensitivity. Intriguingly, overexpression of human precursor-let-7 miRNAs led to differential RRM2 expression and gemcitabine chemosensitization in a poorly differentiated gemcitabine-resistant pancreatic cancer cell line MIA PaCa-2. Defective processing of the let-7a precursors to mature forms explained, in part, the discrepancies observed with let-7a expressional outcomes. Screening putative regulators of let-7a biogenesis in MIA PaCa-2 cells identified LIN-28 and SET oncoprotein to differentially modulate let-7 biogenesis and chemosensitivity in gemcitabine-sensitive versus-resistant pancreatic cancer cells. Additionally, acquired gemcitabine chemoresistance was found to be independent of let-7 alterations. These data demonstrate an intricate post-transcriptional regulation of RRM2 expression by let-7 and that the manipulation of let-7 processing machinery may provide a mechanism for improving gemcitabine chemosensitivity in innately-resistant pancreatic cancer cells.

A32 Decoding the functional role of noncoding RNAs. Coby B. Carlson[1], Lesslie Beauchamp[2], Susan Magdaleno[2], David Piper[1]. [1]Life Technologies, Madison, WI, [2]Life Technologies, Austin, TX.

Less than 2% of the human genome actually encodes protein sequences. The remaining untranslated or non-coding RNA (ncRNA) has been historically disregarded as transcriptional "noise". There is increasing evidence to suggest, however, that the importance of ncRNAs might mirror that of protein-coding genes. And although the precise function of these molecules is still largely unknown, it may not be surprising that the dysregulation of ncRNA expression appears to be a primary feature of many complex human diseases.

In an effort to better understand the functional role of various ncRNAs, we have employed a complimentary set of genomic tools and cellular assay research technologies. Using a library of Silencer® Select siRNA oligos designed against a validated set of 45 known ncRNA sequences, we evaluated the effects of target-specific knockdown via RNAi on various signal transduction pathways within a panel of CellSensor® reporter-gene assays. This has enabled us to create an activity profile for these ncRNAs by analyzing them with the same assay readout in different cell backgrounds (e.g., analysis of Wnt signaling in HCT-116 vs. SW480 cells), as well as comparing their effects on different signaling pathways within the same cell background (e.g., Notch signaling vs. heat shock response). The positive hits from these screens were evaluated further using TaqMan® non-coding RNA assays and related cell-based assays. Many interesting findings have surfaced and we are currently sorting out what the actual roles are (or might be) for many of these transcripts. This discovery-based approach that uses tools from our company – providing a novel offering that is unavailable anywhere in the world today – should greatly advance the understanding of how ncRNAs impact cell regulatory and disease mechanisms.

"For Research Use Only. Not for human or animal therapeutic or diagnostic use."

A33 The role of microRNA-181a in TGF-β-mediated breast cancer progression. Molly A. Taylor[1], William P. Schiemann[2]. [1]Case Western Reserve University, Cleveland, OH, [2]Case Comprehensive Cancer Center, Cleveland, OH.

Transforming growth factor-beta (TGF-β) is a multifunctional cytokine that functions to inhibit breast cancer development in the normal mammary epithelium by inducing cell cycle arrest, and maintaining microenvironmental homeostasis through extracellular matrix (ECM) deposition and remodeling. Mammary tumorigenesis causes a loss in TGF-β-mediated cytostasis and leads to a switch in TGF-β function, whereby TGF-β promotes epithelial-mesenchymal transition (EMT), proliferation, invasion and metastasis. This switch in TGF-β function, from tumor suppressor to tumor promoter, is referred to as the "TGF-β paradox" and occurs through molecular mechanisms that remain incompletely understood. Moreover,

this switch in TGF-β function is often accompanied by desmoplastic and fibrotic reactions, leading to the formation of a more rigid microenvironment, which in and of itself has been associated with tumor progression. We have shown that tissue compliance and extracellular matrix rigidity mediate how cells sense and respond to TGF-β. Indeed, we can recapitulate the "TGF-β paradox" by exposing metastatic breast cancer cells to compliant ECM signals, which reinstates the cytostatic activities of TGF-β. As a possible mechanism for the influence of matrix rigidity on TGF-β signaling, we sought to define the miRNA expression profiles induced by TGF-β in metastatic cells as compared to their non-metastatic counterparts when grown in either compliant or rigid conditions. We show that ECM rigidity regulates the miRNA expression signature induced by TGF-β in breast cancer cells. From this miRNA signature, we have identified the miR-181 family as a miR family that is highly upregulated in metastatic cells in response to TGF-β. Additionally, over-expression of a miR-181a mimic enhances TGF-β-mediated invasion. Conversely, treating cells with a hairpin inhibitor directed against miR-181a inhibits TGF-β-mediated invasion, EMT, and survival signaling. Furthermore, inhibition of miR-181a abrogates pulmonary outgrowth and enhances overall survival in vivo. Collectively, our findings identify the miR-181a family as a novel effector of TGF-β-driven tumor progression and suggest that measures capable of diminishing miR-181a levels may alleviate breast cancer progression.

[FY09-DOD-BCRP-Predoctoral Traineeship Award].

Poster Session B

B1 microRNA-424 as a potential regulator of mammary cell epithelial-to-mesenchymal transition and breast cancer stem-like cell properties. David J. Drasin, Deepika Neelakantan, Heide L. Ford. University of Colorado Anschutz Medical Campus, Aurora, CO.

The epithelial-to-mesenchymal transition (EMT) is a cellular program in which epithelial cells lose epithelial characteristics and gain mesenchymal characteristics, resulting in a range of acquired traits such as increased plasticity that allow stationary epithelial cells to become motile. This process is necessary for proper development during embryogenesis and for wound healing later in life. A similar aberrant, and more transient, EMT program can contribute to the metastatic dissemination of epithelial cancer cells, and is referred to as an oncogenic EMT. Recent breast cancer studies have identified components of the microRNA-200 family as major players in maintaining the epithelial phenotype of cancer cells; the loss of the microRNA-200 family coincides with more aggressive breast cancers in humans. We find that through the course of an induced EMT in human mammary cells, mesenchymal programming is activated prior to epithelial gene repression and prior to loss of members of the microRNA-200 family; however, epithelial genes are ultimately decreased. This simultaneous expression of mesenchymal and epithelial proteins at this early time point, as opposed to an all or none phenotype, may more closely resemble changes seen in human carcinomas. To identify microRNAs important during this early mesenchymal-like transition, we performed a microRNA microarray screen. We identified multiple oncogenic EMT inducers as up-regulating microRNA-424 concomitantly with the induction of a mesenchymal-like phenotype, and before the repression of epithelial markers and the microRNA-200 family. Overexpression of microRNA-424 in non-transformed human mammary epithelial cells is sufficient to produce a mesenchymal-like phenotype, as well as increase the mammary stem cell population in vitro. These data suggest that microRNA-424 may play an important role in both the mesenchymal programming and increased tumor-initiating cell formation that occur during an oncogenic EMT. Further investigation of microRNA-424 may yield new targets for inhibiting EMT and/or tumor-initiating cell formation during breast cancer.

B2 Treatment of ovarian cancer with targeted tumor-penetrating siRNA nanocomplexes. Yin Ren[1], Hiu Wing Cheung[2], Ronny Drapkin[3], David Root[2], Justin Lo[4], Valentina Fogal[5], Erkki Ruoslahti[5], William Hahn[2], Sangeeta Bhatia[4], Geoffrey von Maltzahn[1], Amit Agrawal[1], Glenn Cowley[2], Barbara Weir[2], Jesse Boehm[2], Pablo Tamayo[2], Jill Mesirov[2], Alison Karst[3]. [1]Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA, [2]Broad Institute of Harvard and MIT, Cambridge, MA, [3]Dana Farber Cancer Institute, Boston, MA, [4]MIT, Cambridge, MA, [5]Burnham Institute for Medical Research, Santa Barbara, CA.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR5) of the conference *Proceedings*.

B3 Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. Ivan Garcia-Bassets. University of California, San Diego, La Jolla, CA.

Mammalian genomes are populated with thousands of transcriptional enhancers that orchestrate cell-type-specific gene expression programs, but how those enhancers are exploited to institute alternative, signal-dependent transcriptional responses remains poorly understood. Here we present evidence that cell-lineage-specific factors, such as FoxA1, can simultaneously facilitate and restrict key regulated transcription factors, exemplified by the androgen receptor (AR),

to act on structurally and functionally distinct classes of enhancer. Consequently, FoxA1 downregulation, an unfavorable prognostic sign in certain advanced prostate tumors, triggers dramatic reprogramming of the hormonal response by causing a massive switch in AR binding to a distinct cohort of pre-established enhancers. These enhancers are functional, as evidenced by the production of enhancer-templated non-coding RNA (eRNA) based on global nuclear run-on sequencing (GRO-seq) analysis, with a unique class apparently requiring no nucleosome remodeling to induce specific enhancer-promoter looping and gene activation. GRO-seq data also suggest that liganded AR induces both transcription initiation and elongation. Together, these findings reveal a large repository of active enhancers that can be dynamically tuned to elicit alternative gene expression programs, which may underlie many sequential gene expression events in development, cell differentiation and disease progression.

B4 microRNAs differentially expressed in preoperative human serum of ovarian cancer. Angel Chao[1], Tzu Hao Wang[1], Hua-Chien Chen[2], Shu-Jen Chen[2], Chyong-Huey Lai[1]. [1]Chang Gung Memorial Hospital, Guishan, Taiwan, [2]Chang Gung University, Guishan, Taiwan.

MicroRNAs (miRNAs) have been reported to play important regulatory roles in many biological processes and tumorigenesis. To investigate the roles of serum miRNAs in patients before tumor bulk was surgically removed from ovarian cancer patients, we utilized miRNA real-time QPCR profiling technology to characterize the miRNAs in pre-operative and postoperative sera. Six pairs of sera were analyzed using 270 miRNA SyberGreen probes. Upregulation of miR187, miR499-3p, miR548c-5p, miR1, miR107, miR125a-5p, miR18b were identified in the preoperative sera. Targetscan was utilized to identify Dab2 as the target gene of miR187. In vitro studies of SKOV3 cells showed that ectopic expression of miR187 suppressed Dab2 and resulted in increased cell proliferation. We concluded that miR187 may be a potential biomarker in sera of ovarian cancer patients. Further studies with larger sample sizes are warranted to validate our results.

B5 A comprehensive noncoding RNA map in lung, liver, and breast cancer uncovers novel players in tumorigenesis. Maria Polycarpou-Schwarz[1], Sebastian Aulmann[2], Thomas Longerich[2], Joachim Rom[2], Thomas Muley[3], Michael Meister[3], Heike Zabeck[3], Peter Sinn[2], Philipp A. Schnabel[2], Hans Hoffmann[3], Peter Schirmacher[2], Tony Gutschner[1], Sven Diederichs[1], Stefanie Grund[1], Monika Hämmerle[1], Anna Roth[1], Julia Neugebauer[1], Evgenij Fiskin[1], Catherina Hildenbrandt[1], Arne Warth[2]. [1]German Cancer Research Center (DKFZ) & Institute of Pathology, Heidelberg, Germany, [2]University Hospital, Heidelberg, Germany, [3]Thoraxklinik, Heidelberg, Germany.

Beyond proteins, the human genome contains many more functionally important and clinically informative entities: the non-coding RNAs (ncRNA). However, for most long ncRNAs, neither their function nor their expression patterns in health and disease are known.

Here, we have discovered novel, important ncRNAs in three major tumor entities that could be active players in tumorigenesis or serve as tumor biomarkers. We profiled the expression of 17000 lncRNAs in lung, breast and liver cancer as well as normal tissues from the respective organs (in total: N=150) using a microarray platform. This expression landscape was complemented with a profiling study of a cell line model for chemotherapy response: lung and liver cancer cells were treated with the DNA-damaging cytotoxic agents cisplatin and etoposide and ncRNA expression patterns were established.

This comprehensive expression map revealed significantly regulated ncRNAs for all parameters tested: besides tissue-specific ncRNAs, these studies also identified prognostic signatures and tumor-specific ncRNAs which were validated in two independent patient cohorts. In addition, ncRNAs associated with the DNA damage response were unraveled that affect tumor cell viability.

As one functional example, a novel transcript that we called “Lung Cancer intergenic RNA 1” (LuCaiR1) is strongly induced in lung adenocarcinoma and its loss impacts the viability of lung cancer cells. LuCaiR1 is also downregulated upon DNA damage in lung cancer cells reversing the tumor-associated deregulation. To study loss-of-function phenotypes in human lung cancer cells, we have developed a novel technique to create functional knockouts of ncRNAs in human cell lines using Zinc Finger Nucleases (ZFN).

To elucidate the molecular functions of the novel ncRNAs, we have used RNA affinity purification to identify the protein interaction partners of the tumor-associated ncRNAs. In one such network, we have identified regulators of the Actin cytoskeleton as major interaction partners for a novel, cytoplasmic breast cancer-induced ncRNA MaCaiR1 (Mamma Carcinoma intergenic RNA 1).

In summary, we provide the first global comprehensive map of long ncRNA expression in a broad range of human tumor and normal tissue samples and discovered many new lncRNAs associated with three major tumor entities. Initial functional characterization has already revealed their functional importance at the cellular level as well as provided hints towards their molecular functions.

B6 Human genome contains a large number of very long intergenic transcribed regions, some of which are associated with cancer outcome. Anirban P. Mitra[1], Timothy J. Triche[2], Sheetal A. Mitra[2], Jonathan D. Buckley[1], Poul H. B. Sorensen[3], C Patrick Reynolds[4], Robert J. Arceci[5], Patrice Milos[6], Georges St. Laurent[7], Philipp Kapranov[7]. [1]University of Southern California, Los Angeles, CA, [2]Children’s Hospital Los Angeles, Los Angeles, CA, [3]University of British Columbia, Vancouver, BC, Canada, [4]Texas Tech University, Lubbock, TX, [5]Johns Hopkins University, Baltimore, MD, [6]Pfizer Center for Therapeutic Innovation, Boston, MA, [7]The St. Laurent Institute, Cambridge, MA.

Introduction: Transcriptional output of human genome is far more complex than predicted by the current set of protein-coding annotations. However, the fraction of the genome that is utilized to produce cellular RNA whose function is hitherto not completely defined, the so called “dark matter RNA”, and its role in regulating cancer progression remains an open issue. Furthermore, our understanding of the repertoire of human RNAs is far from complete, and almost all RNA-Seq studies have missed this complexity due to the limited view obtained when only interrogating the polyA+ RNA fraction confounded by biases due to PCR amplifications. This study aimed to assess the complexity of RNAs produced by cancerous and normal human tissues in an unbiased fashion, and examine any potential roles of such transcripts in modulating cancer outcome using pediatric rhabdomyosarcoma as a model malignancy.

Methods: Total RNA was profiled from a number of tissues using single-molecule sequencing (SMS) to examine the complexity of transcriptome in the human genome in an unbiased fashion. To minimize methodological biases, no PCR amplification, ligation, or size selection were used. In parallel, to examine the relevance of different RNAs in predicting cancer outcome, expression profiles on 79 intermediate-risk childhood rhabdomyosarcoma primary tumors were generated on Affymetrix Human Exon 1.0 ST microarrays and compared to prospectively-

obtained outcome information.

Results: SMS analysis revealed that "dark matter RNAs" comprised up to two-thirds of total non-ribosomal, non-mitochondrial RNA in human cells. PolyA+ RNA fraction had a significantly lower complexity then the total RNA, especially in non-exonic regions. Strikingly, several hundreds of very long (100's of kbs) abundant intergenic transcribed regions (vlinc's) were identified in areas of the genome that were devoid of protein-coding annotations. Most (~80%) of the genomic sequences covered by vlincs did not overlap with previously documented long intergenic non-coding (linc) RNAs, suggesting large number of RNAs in intergenic space are yet to be uncovered. To address the question whether vlincs may be associated with prognosis in cancer, specifically childhood rhabdomyosarcoma, array-based whole-genome expression profiles were analyzed. Interestingly, probe sets that were the best predictors of cancer outcome were found to be outside boundaries of exons of protein-coding transcripts. Strikingly, most of the top-ranked probe sets were found to cluster together and defined a >230 kb vlinc region on chromosome 2, previously found by the SMS analysis. This vlinc region was singularly able to predict survival for the entire cohort (p=0.007).

Conclusions: Our data suggest that many genomic regions currently defined as intergenic give rise to very long transcribed regions that can modulate ultimate cancer behavior. This indicates that a great number of the "dark matter" RNAs uncharacterized thus far may be involved in tumorigenesis, and can be used as diagnostic, prognostic and therapeutic-response indicators. In turn, this argues for functional importance of the genome's "dark matter".

B7 microRNA regulation of cell viability and drug sensitivity in lung cancer. Liqin Du[1], Christopher DeSevo[2], Borkowski Robert[2], Baker Michael[2], Adi F. Gazdar[2], John D. Minna[2], Alexander Pertsemlidis[1]. [1]UT Health Science Center at San Antonio, San Antonio, TX, [2]UT Southwestern Medical Center, Dallas, TX.

Introduction: Lung cancer is the leading cause of cancer-related deaths, with the majority of deaths due to failed therapy from tumor drug resistance. Third-generation chemotherapeutic agents represent the standard first-line treatment for advanced small cell (SCLC) and non-small cell (NSCLC) lung cancer patients. Response rates are poor (20-40%) with a median survival of 8–10 months.

Methods: In an unbiased and comprehensive approach, we have combined a high-throughput screening platform with a library of chemically synthesized microRNA mimics and inhibitors. We have used this platform to identify mimics and inhibitors that reduce cell viability in general, and those that specifically sensitize cells to taxanes.

Results: We have identified several miRNAs for which over-expression or inhibition has a dramatic and selective effect on cell viability or drug response. We have demonstrated that miR-337-3p mimic sensitizes NSCLC cells to taxanes. By combining in vitro and in silico approaches, we identified STAT3 and RAP1A as direct targets that mediate the effect of miR-337-3p by enhancing taxane-induced arrest in the G2 phase of the cell cycle. We have also identified an inhibitor of miR-139-5p as a potent and selective regulator of SCLC cell viability. Inhibiting miR-139-5p decreases SCLC cell viability by over 80%, but has a minimal cytotoxic effect on that of NSCLCs or immortalized human bronchial epithelial cells. We are currently investigating the targets of miR-139-5p that mediate its effect on SCLC cell viability.

Conclusion: We have identified miR-337-3p as a regulator of taxane sensitivity in NSCLC, and miR-139-5p as a modulator of viability in SCLC. Increasing levels of miR-337-3p and inhibiting miR-139-5p may therefore provide novel therapeutic tools for the treatment of NSCLC and SCLC lung tumors, respectively.

B8 Molecular function of the RNA binding protein EWS in RNA processing. Bethsaida I. Nieves, Shuang Niu, Dedeepya Vaka, Julia Salzman, Patrick Brown, E. Alejandro Sweet-Cordero. Stanford University School of Medicine, Stanford, CA.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR4) of the conference *Proceedings*.

B9 Critical early microRNA-mediated signaling events during ATRA induced neuroblastoma cell differentiation. Niamh H. Foley, Isabella M. Bray, Raquel Fernandez, Raymond L. Stallings. Royal College of Surgeons in Ireland, Dublin, Ireland.

Introduction: Neuroblastoma (NB) is a childhood cancer arising from precursor cells of the sympathetic nervous system with highly heterogeneous clinical behavior, ranging from spontaneous regression to rapid progression and death due to disease. Amplification and over-expression of the MYCN transcription factor in neuroblastoma tumors is highly correlated with poor survival of patients. Children with high risk NB are given the synthetic retinoic acid, 13-cis-retinoic acid, to reduce minimal residual disease, and exposure of a number of NB cell lines to the related compound, all-trans-retinoic acid (ATRA), induces neural differentiation along with the down-regulation of MYCN. We previously determined that miRNA-10a/b plays a critical role in the process of ATRA induced neuroblastoma cell differentiation through direct targeting of nuclear co-repressor receptor 2 (NCOR2) (Foley et al Cell Death & Differentiation 2011: 18:1089). Here, we have profiled three ATRA sensitive (SK-N-BE, LAN5 and SHSY-5Y) and two ATRA resistant (Kelly and SK-N-AS) neuroblastoma cell lines for the expression of 750 miRNA loci at early (3 hours) and late (7 days) time points following ATRA treatment. The purpose of our experiments was to identify critical early miRNA signaling events, as well as miRNAs that might mediate sensitivity versus resistance to ATRA.

Methods: All cells were treated with 5 μmol ATRA (Sigma, St. Louis, MO) which was continuously administered by replacing the medium every 24 hrs. MiRNA expression profiling was carried out using TaqMan Low Density Arrays from Applied BioSystems (Carlsbad, CA).

Results: miR-10a, -34a, -125b, -28-5p, and -183 were consistently up-regulated and miR-450-5p, -151-3p and -138-2 were consistently down-regulated >2 fold ($p < 0.05$) in all three ATRA sensitive cell lines at three hours post-ATRA. At 7 days post-ATRA, there were more differentially expressed miRNAs, with 13 miRNAs being up-regulated and 18 down-regulated > 2 fold ($p < 0.05$) across all three cell lines. Among the miRNAs differentially expressed at 3 hours post-ATRA, miR-10a, miR-125b and miR-34a remained differentially expressed at 7 days post-ATRA. Remarkably, none of the differentially expressed miRNAs in the ATRA sensitive cell lines were differentially expressed in the ATRA resistant cell lines at the three hour time point, and very few displayed altered expression in the ATRA resistant cell lines even after a 7 day exposure to ATRA. qPCR and Western blot analysis of the critical miR-10a target, NCOR2, revealed that this gene is down regulated at both mRNA and protein level at the 3 hour time point. In addition to the targeting of NCOR2 by miR-10a, we noted that two additional up-regulated miRNAs, miR-125b and miR-132 (significantly up-regulated within 7 days post-ATRA) are also predicted to target NCOR2, indicating substantial redundancy in miRNA mediated signaling. Direct targeting of NCOR2 by these additional miRNAs was experimentally validated by luciferase reporter assays. This redundancy in miRNA targeting explains why antagomir inhibition of single miRNAs prior to ATRA treatment only delays, but does not block the induction of differentiation. Finally, we present evidence that an initial early decrease in MYCN levels, which is well known to occur in neuroblastoma cells treated with ATRA, is miRNA mediated.

Conclusions: We have identified critical and redundant early miRNA signaling events that take place during the process of ATRA induced neuroblastoma cell differentiation. The absence of these events in ATRA resistant cell lines suggests new avenues of

investigation for elucidating potential miRNA mediated mechanisms involved with ATRA therapy failure.

B10 Genome-wide profiling reveals hyper- and hypomethylation at microRNA promoters in chronic lymphocytic leukemia. Constance Baer[1], Michael Rehli[2], John C. Byrd[3], Clemens-Martin Wendtner[4], Christoph Plass[1], Rainer Claus[1], Lukas Frenzel[4], Manuela Zucknick[1], Yoon Jung Park[1], Lei Gu[1], Dieter Weichenhan[1], Martina Fischer[1], Christian Pallasch[4]. [1]German Cancer Research Center (DKFZ), Heidelberg, Germany, [2]University Hospital Regensburg, Regensburg, Germany, [3]The Ohio State University, Columbus, OH, [4]University Hospital of Cologne, Cologne, Germany.

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world. Dysregulation of microRNA (miRNA) expression contributes to CLL pathogenesis. Abnormal promoter methylation is involved in aberrant gene expression in leukemic cells. Here, we investigate the role of epigenetic mechanisms in the global regulation of miRNA expression in CLL.

In order to characterize disease-specific aberrant methylation upstream of miRNAs, methylated DNA from 24 CLL patients and 10 healthy age-matched controls was enriched by Methyl-CpG immunoprecipitation. Differentially methylated genomic loci were identified using a custom tiling array covering the genomic sequences 35 kb upstream and 5 kb downstream of 939 annotated miRNAs. We identified putative promoter sequences for 781 miRNAs by determining regions of enriched trimethylated histone 3 lysine 4 (H3K4me3) in CLL samples, healthy B cells or cancer cell lines.

The methylation patterns upstream of miRNAs clearly distinguished CLL cells from healthy B cells. Differentially methylated sequences are enriched in H3K4me3, suggesting that miRNA promoters are non-randomly targeted for epigenetic dysregulation. 458 miRNAs display differential methylation in at least 5 out of the 24 CLL samples. Noteworthy, hypomethylation accounted for two thirds of all differentially methylated regions at putative miRNA promoters. Hypomethylated regions are limited to strictly defined sequence stretches, predominantly found outside of CpG islands (89%).

Correlating promoter methylation with miRNA expression, we observed loss of methylation and strong transcriptional upregulation of mir-155 in CLL. Similarly, the promoter of mir-1204 showed significantly reduced methylation in CLL associated with increased expression. Mir-129-2, a well known tumor suppressor in solid tumors, could be detected among the epigenetically silenced miRNAs. Furthermore mir-551b, which has not yet been described in the context of CLL, was found to be downregulated and a target of promoter hypermethylation in CLL cells.

In conclusion, a combination of DNA methylation profiling and comprehensive miRNA promoter identification has allowed us to identified novel aberrantly regulated miRNAs in CLL. Hypomethylation, which has previously been underestimated, is determined to be a major mechanism for miRNA activation in CLL.

B11 Expression of microRNAs-135b and -196b in response to cellular stress in leukemia cells. Tsui-Ting Ho, Xiaolong He, Ahmet Dirim Arslan, William T. Beck. University of Illinois at Chicago, Chicago, IL.

MicroRNAs are a class of non-coding small RNA molecules that have roles in important biological processes. Multidrug resistance is a key obstacle to successful cancer therapy in human cancers. To determine whether microRNAs have a role in the development or maintenance of multidrug resistance, we used the TaqMan™ Human MicroRNA Array to investigate the expression of microRNAs in multidrug resistant (MDR) CCRF-CEM human T-cell leukemia cells (CEM/VM-1-5) and drug-sensitive parental line, CCRF-CEM (CEM). The MDR line was selected for resistance to teniposide and is cross-resistant to etoposide and other drugs. Our profiling study of 365 microRNAs revealed that certain microRNAs are differentially expressed between CEM/VM-1-5 and CEM cells, and we focused our

subsequent work on two of these: miR-135b and -196b. Since MDR cells were selected and developed by stepwise increases in concentrations of teniposide, we therefore asked whether these altered microRNAs in MDR CEM/VM-1-5 cells are associated with cellular responses to genotoxic agents. We observed substantial increases in miR-135b and -196b expression in drug-sensitive CEM cells following their short exposure (3 to 48 hours) with etoposide, doxorubicin and topotecan, but not in cells treated with vinblastine or paclitaxel, suggesting that the upregulation of these two microRNAs might be the consequence of DNA damage. We also found similar increases in miR-135b and -196b in other leukemia cell lines (RPMI 8226 [myeloma], HL-60 [acute promyelocytic leukemia], Jurkat and MOLT-4 [both acute T cell leukemia]), but not in solid tumor-derived cell lines (MCF7 breast cancer and A2780 ovarian cancer). This suggests that induction of expression of these microRNAs is histiotype-specific. To further confirm that upregulation of these two microRNAs is a consequence of DNA damage, we examined expression of miR-135b and -196b following ionizing radiation. When we treated CEM pairs with ionizing radiation, MDR CEM/VM-1-5 cells were more radioresistant compared to CEM cells. As expected, higher doses of radiation induced miR-135b and -196b expression in MDR CEM/VM-1-5 cells compared to CEM cells, in accordance with what we observed in cells treated with the DNA damaging agent etoposide. Moreover, time-course and dose-response studies showed that the elevation of miR-135b and -196b correlated positively with the exposure time and radiation dosage. Together, our results suggest that microRNAs might play a significant role in cellular response to genotoxic agents and upregulation of miR-135b and -196b appears to be a marker of DNA damage in leukemia cells.

(Supported in part by RO1CA40570 from NCI to WTB, and in part by UIC.)

B12 The miR-15 family and miR-155 mediate WEE1 and CHEK1 effects on cisplatin resistance in cancer cells. Lynn M. Pouliot[1], Jennifer Bai[1], Matthew D. Hall[1], Scott E. Martin[2], Michael M. Gottesman[1]. [1]National Cancer Institute, Bethesda, MD, [2]National Human Genome Research Institute, Rockville, MD.

A major challenge in the successful treatment of cancer is the ability of cancer cells to acquire a multi-drug resistant (MDR) phenotype, preventing response to chemotherapeutic agents. Cisplatin (cis-diamminedichloroplatinum(II)) is a chemotherapeutic drug commonly used against malignancies including ovarian, and cervical tumors. Cisplatin resistance (CP-r) studied in cultured cancer cells often results from a cellular defense mechanism that results in a highly complex pleiotropic phenotype that confers resistance by reducing apoptosis, up-regulating DNA damage repair mechanisms, altering cell cycle checkpoints, altering protein localization, redirecting cell surface transporters, and disrupting assembly of the cytoskeleton thereby changing protein distribution in the cell. This results in cells that are permanently resistant not only to cisplatin but also show cross-resistance to multiple toxic compounds that piggyback into cells on existing transporters. The highly pleiotropic CP-r phenotype occurs in a single step that has yet to be identified.

miRNAs have crucial roles in diverse biological processes such as phenotypic stabilization, and have been shown to also be involved in tumor growth and response to chemotherapy. We hypothesized that miRNAs may play a role in mediating the phenotypic changes associated with a pleiotropic cisplatin resistance mechanism.

KB-CP.5 (resistant to 0.5ug/ml CP) cells were screened with whole kinome siRNA (Ambion SilencerSelect, 3 siRNAs per gene) and miRNA mimics (Sanger miRBase 13.0). Cells were then challenged with cisplatin at IC5 and IC30 doses (normally causing minimal toxicity to CP-r cells). Cell viability was determined using the Promega CellTiter-Glo luminescent viability assay, and conditions that sensitized the CP-r cells were examined. The miR-15/16/195/424/497 family miRNA mimics were

found to confer >2-fold increased sensitivity to CP.5 cells. miR-16 gave a 5-fold increase in sensitivity and its family member miR-15a caused a 3.4-fold increase, accounting for two of the top three hits in the primary screen. Follow-up dose-response analysis indicated that miR-15a and miR-16 mimics yielded a 20- and 10-fold shift in the cisplatin sensitivity of CP.5 cells, respectively. The two strongest kinome effects were elicited by silencing WEE1 and CHEK1. Using TargetScanHuman (available at www.targetscan.org), an algorithm program for the prediction of miRNA targets, we found that the miR-15 family is predicted to target WEE1 and CHEK1. For the miR-15 family, RT-PCR shows a 2 to 5-fold decrease in expression in the CP-r cells compared to KB-3-1 parental cells. In addition miR-155 has a 3-fold decrease in expression in KB-CP.5 compared to KB-3-1 parental cells. TargetScan analysis of WEE1 indicates that miR-155 is also a potential regulator of the WEE1 kinase. We confirmed the ability of the miR-15 family and miR-155 to sensitize resistant cells to cisplatin by targeting WEE1 and CHEK1 by performing loss-of -function and gain-of-function experiments. Using the MTT cytotoxicity assay and full cisplatin dose response curves, miRNA inhibitors of the miR15 family increased resistance while mimics sensitized resistant cells. miR-155 mimics resulted in sensitivity returning to parental levels and inhibitors of WEE1 and CHEK1 sensitized KB-CP.5 cells to CP.

In conclusion, we have identified a miRNA family responsible for regulating the cell-cycle checkpoint kinases WEE1 and CHEK1 associated with cisplatin resistance.

B13 Automated multiplex assay for diagnostic application of contextual microRNA signatures in breast cancer. Wendy Wells, Sung-hee Choi, Gary Schwartz, Lorenzo F. Sempere. Dartmouth-Hitchcock Medical Center, Lebanon, NH.

MicroRNAs (miRNAs) are rapidly emerging as a novel class of biomarkers for theranostic applications in cancer. Since solid tumor lesions are complex and heterogeneous mixtures of different cell types, we previously developed a multiplex tissue slide-based assay to measure miRNA expression levels at single cell resolution and to extract the contextual information embedded within both cancer and non-cancer cell compartments of the tumor microenvironment.

In this on-going clinical study, we are characterizing the cell type-specific altered expression of a panel of breast cancer-associated miRNAs on large and independent cohorts of breast cancer patients. To maximize the number of independent patient cases per slide and to minimize the number of independent experiments and slides for data analysis, we are interrogating miRNA expression on high-density tissue microarrays (TMAs). TMAs designed by NCI Cooperative Breast Cancer Tissue Resources to find statistically significant associations between an investigative biomarker and disease progression and/or disease outcome will serve as discovery set. Our institutional TMAs will serve as validation set and will also enable us to assess the potential utility of these miRNA contextual signatures to predict treatment response in different adjuvant and neoadjuvant therapy settings using recurrence-free survival and complete pathological response, respectively, as the clinical endpoint marker.

Our preliminary results indicate that inflammation and desmoplasia present as co-diseases or co-morbidities in breast cancer, and they are both major confounding factors in the correct interpretation of expression profiling experiments and the assignment of etiological roles to leukocyte-expressed miRNAs such as miR-155 and reactive fibroblast-expressed miRNAs such as miR-21. Intriguingly, we have identified a subgroup of HER2-overexpressing cases in which miR-21 expression is mainly upregulated with cancer cells, while miR-21 expression predominantly accumulates within reactive fibroblasts in the rest of HER2-overexpresssing cases and the immense majority of cases from other breast cancer subtypes. The clinical implications of cell source and expression levels of miR-21 and other miRNAs under study will be presented at this meeting.

We will also discuss innovative strategies that we are implementing for integration of translational miRNA and well-established

protein biomarkers to increase molecular characterization of breast tumors and for pipeline automatization from assay performance to whole slide image acquisition and computer-assisted analysis to enhance diagnostic power of current clinical assays.

B14 Anti-miR-135b in colon cancer treatment. Nicola Valeri[1], Roberta Gafa'[2], Gerard Nuovo[1], Giovanni Lanza[2], Wendy Frankel[1], Peter K. Vogt[3], Joanna Groden[1], Michael Karin[4], Carlo M. Croce[1], Chiara Braconi[1], Pierluigi Gasparini[1], Sergei Grivennikov[4], Jonathan R. Hart[3], Alessio Paone[1], Francesca Lovat[1], Muller Fabbri[1]. [1]The Ohio State University, Columbus, Ohio, [2]University of Ferrara, Ferrara, Italy, [3]The Scripps Research Institute, La Jolla, CA, [4]UCSD, La Jolla, CA.

Background: MicroRNAs (miRs) are small non coding RNAs involved in cell homeostasis. miRs are deregulated in colorectal cancer (CRC). Our study aimed at identifying miRs with a driver role in carcinogenesis altered by similar mechanisms in both human and mouse CRC. Goal of the study was to use CRC mouse models for the pre-clinical development of anti-miRs as therapeutic drugs. Methods: Azoximetane (AOM)/ Dextran-Sulfate (DSS) treated mice or CDX2Cre-APC f/wt mice were used to study inflammation-associated and sporadic APC-related CRC. Human Inflammatory Bowel Disease associated (n=30), and sporadic (n=90) CRC with their matched normal tissues were collected according to Good Clinical Practice recommendation and subjected to RNA extraction using Trizol. miR and gene expression profiling was assessed by nCounter technology (Nanostring Seattle). AntimiR-135b and scrambled probes for in vivo studies were synthesized by Girindus. Results: miRs profiling from AOM/DSS and CDX2Cre-APC f/wt CRC. revealed that miR-135b is one of the most up-regulated miRs in both models. In humans miR-135b over-expression was found in both IBD and sporadic CRC and was associated with reduced Progression Free Survival and Overall Survival in CRC patients. Molecular studies in Mouse Embryo Fibroblast and human CRC cell lines highlighted the role of two major pathways in the upstream activation of miR-135b: APC-β-Catenin and SRC-PI3K. MiR-135b up-regulation resulted in reduced apoptosis and increased invasion and metastasis due to the down-regulation of TGFRB2, DAPK1, APC and HIF1AN. Silencing of miR-135b in vivo reduced tumor multiplicity and tumor load in the AOM/DSS CRC model. Mice treated with anti-miR-135b showed well differentiated tumors and acinar pattern while tumors in the control groups showed low differentiation and adenomatous pattern. Conclusions: Our data suggest that miR-135b is a key molecule whose activation is downstream of oncogenes and oncosuppressor genes frequently altered in CRC. Our study defines specific pathways that converge on the activation of the same microrna. The "in vivo" silencing of miR-135 shows preclinical efficacy with low toxicity and represents the first in vivo study for the use of antimiRs in CRC treatment

B15 Repression of PDGFRA-targeting miR-34a promotes tumorigenesis in proneural malignant gliomas. Joachim Silber, Anders Jacobsen, Tatsuya Ozawa, Girish Harinath, Eric C. Holland, Chris Sander, Jason T. Huse. Memorial Sloan-Kettering Cancer Center, New York, NY.

Malignant gliomas - particularly glioblastoma (GBM) - continue to cause a disproportionate degree of morbidity and mortality within human oncology. Recent integrated genomics has demonstrated biologically distinct subclasses within malignant glioma that transcend conventional histopathological boundaries. Perhaps the broadest of these subclassess has been termed "proneural" and includes both a large fraction of GBMs along with most of their lower-grade astrocytic and oligodendroglial counterparts. The pathogenesis of proneural gliomas has been strongly linked to dysregulated PDGF signaling; but although genomic amplification or activating mutations involving the PDGF receptor (PDGFRA) characterize a significant subset of proneural GBMs, the mechanisms driving dysregulated PDGF signaling and downstream oncogenic networks in non-amplified/mutated tumors remain unclear. MicroRNAs (miRNAs) are a

class of small, noncoding RNAs that regulate gene expression on a pre-translational level by binding loosely to complimentary sequences in target mRNAs. The role of miRNA biology in numerous cancer variants is well established. In an analysis of miRNA involvement in the phenotypic expression and regulation of oncogenic PDGF signaling, we found that expression of miR-34a is responsive to PDGF pathway activation in vitro. Similarly, analysis of data from the Cancer Genome Atlas (TCGA) revealed that expression of miR-34a is highly negatively correlated in proneural gliomas and in gliomas harboring amplified PDGFRA compared to other tumor subtypes. Using primary GBM cells maintained under neurosphere conditions, we then demonstrated that miR-34a specifically affects growth of proneural GBM cells in vitro and in vivo. Using data from TCGA, we identified PDGFRA as a direct target of miR-34a and validated this interaction experimentally. Finally, we provided evidence for a p53-independent mechanism mediated by PDGF signaling for negative regulation of miR-34a in proneural tumors. Taken together, our data suggest reciprocal negative feedback regulation of miR-34 and PDGFRA expression in proneural gliomas and, as such, identify a subtype specific therapeutic potential for miR-34a.

B16 The microRNA signature characterizing neuroblastoma cancer stem cells collectively targets the p53 pathway. Danielle Hsu[1], Ashley Benham[2], Zaowen Zaowen[1], Cristian Coarfa[1], Eugene Kim[1], Preethi Gunaratne[3], Jason M. Shohet[1]. [1]Baylor College of Medicine, Houston, TX, [2]Methodist Hospital, Houston, TX, [3]University of Houston, Houston, TX.

We have recently isolated a novel sub-population of neuroblastoma tumor cells with phenotypic and molecular similarities to induced pluripotent stem cells. In addition, we demonstrate that this population is highly tumorigenic, can self-renew, has a stem cell like cell cycle, and recapitulates entire heterogeneous tumors composed of progeny and a small CSC-like population. Disabled p53 function is a common feature of embryonic stem cells and induced pluripotent stem cells (iPSCs) and is likely involved in malignant stem cell biology.

Results: MicroRNA expression profiling of FACS purified sub-populations was performed by small RNA seq and confirmed by Q-PCR assays. We demonstrate a highly consistent signature of 25 up-regulated microRNAs in the CSC-like population. In silico pathway analysis tools demonstrate that 15 of these microRNAs target 21 components of the p53 signaling pathway including 8 apoptosis genes and 6 cell cycle genes with multiple microRNAs targeting the same genes. Importantly, 6 of these microRNAs target p21(CIP/Waf) , a major regulator of the G1/S checkpoint implicated in the regulation of neural stem cell differentiation and down regulated during iPSC regeneration. We demonstrate markedly reduced levels of p21 as well as remarkably consistent stem cell-like cell cycle in neuroblastoma CSC sub-populations. We propose that differential regulation of microRNAs targeting p21 disables the G1/S checkpoint and promotes the maintenance of a distinct CSC sup-population in neuroblastoma.

B17 Inhibition of lung tumor progression in a metastatic mouse model following intravenous delivery of siRNA nanocomplexes. Jason G. Fewell, Khursheed Anwer, Kevin Polach, Majed Matar, Jennifer Rice, Angela Rea-Ramsey, Jeff Sparks, Diane McClure, Casey Pence, Elaine Brunhoeber, Leslie Wilkinson. EGEN, Inc., Huntsville, AL.

Exploiting the RNAi pathway offers the potential to advance the treatment of many diseases through highly specific and efficient silencing of gene products. Unfortunately, the requisite safe and efficient delivery of nucleic acids to target cells remains a fundamental problem for the development of RNA and DNA based therapeutics. We have developed a versatile lipopolyamine based delivery system that has been optimized for in vivo delivery by incorporating functional groups onto the core cationic lipopolyamine structure. The direct modification of the core structure (named Staramine) allows for the generation of nanoparticle formulations

with a wide range of physicochemical properties and does not require the co-formulation of commercial helper or pegylated lipids. We have previously reported safe, efficient and persistent siRNA mediated transcript knockdown specifically in the lung endothelium using the Starmaine delivery system. Here we extend those observations by administering a siRNA that targets vascular endothelial growth factor receptor 2 (VEGFR-2; KDR/Flk-1) in an animal model of metastatic lung cancer. VEGFR-2, a receptor tyrosine kinase, has been shown to be mainly expressed on endothelial cells and plays a critical role in cell proliferation and differentiation and consequently is also important in angiogenesis associated tumor growth and metastasis. Repeated intravenous administration of nanocomplexes comprised of Staramine formulated VEGFR-2 siRNA (~2 mg/kg/dose) resulted in a significant (40%) reduction of VEGFR-2 transcript levels in isolated tumors from the lungs of mice compared to control injected animals. Similar levels of transcript knockdown were not achieved in tumors when using siRNAs against targets not having the high level of endothelial cell restriction that is seen with VEGFR-2. Immunohistopathology of the lungs of tumor bearing mice administered VEGFR-2 siRNA indicated a significant decrease in vascular density in lung tumors and was consistent with an overall reduction in tumor burden in the mouse lungs. These results support the continued evaluation of using Staramine to deliver siRNAs and other similar molecules as potential therapies for diseases of the lung where the vascular endothelium may be involved.

B18 The transcribed noncoding genome modulates the biology of Ewing sarcoma family of tumors. Sheetal A. Mitra[1], Daniel H. Wai[1], William A. May[1], Jonathan D. Buckley[2], Philipp Kapranov[3], Robert J. Arceci[4], Timothy J. Triche[1]. [1]Children's Hospital Los Angeles, Los Angeles, CA, [2]University of Southern California, Los Angeles, CA, [3]The St. Laurent Institute, Cambridge, MA, [4]Johns Hopkins University, Baltimore, MD.

Introduction: Ewing sarcoma family of tumors (EFT) is the second most common primary malignant bone tumor in children and adolescents. It features a characteristic chromosomal translocation; the most common being (t11;22)(q24;q12), which encodes for the chimeric EWS-FLI1 oncoprotein. The disease is highly invasive with a 30% 5-year survival rate for patients with metastatic disease. This study identifies and characterizes a non-coding locus that is unique to EFTs and mediates at least in part the role of the chimeric oncogene in EFT pathogenesis. This particular locus suggests that better understanding of the functional non-coding transcriptome of this developmental cancer can potentially assist in discovering biomarkers that improve the diagnosis and therapeutic management of the disease.

Methods: Expression profiles from a panel of 500 tissue samples including over 100 EFTs, other primary tumors, and normal tissues were generated using Affymetrix Human Exon 1.0 ST microarrays. Data were analyzed using institutional software, Genetrix. RNAseq data was generated on two EFT cell lines derived from a primary tumor and its metastatic derivative. Data was validated by PCR on 33 cell lines including EFTs and other tumors. Functional studies on EFT cell lines were performed using various RNAi techniques and viral transductions. Additional characterization of the RNA was done by cellular fractionation studies and northern blots.

Results: Expression microarray profiles showed unannotated transcripts to be the strongest discriminators of EFTs from all other tumor types. One such transcript, *AK057037*, was the most highly expressed RNA (1.07×10^{-20}) in EFTs. In silico modeling and RNASeq analyses revealed that this

transcript is a multi-exonic, presumably, long non-coding RNA (lncRNA) with a maximum predicted open reading frame of 137 amino acids that had no homology, protein domains, or characteristic motifs. Additionally, multiple variants of the transcript exist in both the nucleus and the cytoplasm. Validation studies confirmed that this lncRNA was exclusively expressed in EFTs. The EFT-specific oncoprotein, EWS-FLI1, regulates expression of this transcript by directly binding to its promoter region. Furthermore, functional data based on knocking down and overexpressing the transcript shows that *AK057037* acts as an oncogene in EFT and enhances tumorigenicity by increasing the metastatic potential of the tumor cell lines.

Conclusion: The study established that lncRNA *AK057037* promotes EFT pathogenesis. Its exclusive presence in EFT suggests that expression of this lncRNA is tissue-specific and highly regulated. Targeting *AK057037* in these tumors may help improve outcomes in children with metastatic disease. Further work on the non-coding transcriptome may reveal additional functional RNAs that contribute to the biology of this disease.

B19 Functional characterization of p53-regulated long intervening noncoding RNAs (lincRNAs). Nadya M. Dimitrova, Tyler Jacks. David H. Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR2) of the conference *Proceedings*.

B20 Breast cancer progression suppressor miR-290 targets breast cancer biomarker Arid4b . Natalie Goldberger, Renard Walker, Chang Hee Kim, Kent Hunter. National Cancer Institute, Bethesda, MD.

Several microRNAs (miRNAs) have been classified as metastasis regulators in breast cancer, yet few studies have examined how germline variations may promote metastasis via miRNA dysregulation. To explore this concept, highly metastatic MMTV-PyMT mice were crossed with 25 AKXD (AKR/J x DBA/2J) recombinant inbred strains to produce F1 progeny with varying metastatic indices. All mammary tumors in the F1 progeny were evaluated by miRNA microarray and correlated with metastatic index to produce miR-290 (containing mature miR-290-3p and miR-290-5p) as one of the top candidates. Further analysis showed miR-290-3p was downregulated in normal lung and breast tissue from highly metastatic AKR/J strains versus less metastatic DBA/2J strains, thereby supporting a germline component to miR-290 regulation. Next, when miR-290 was ectopically expressed in Mvt-1 and 6DT1, and orthotopically injected into FVB/N mice, a significant reduction in both mammary tumor burden and the number of pulmonary metastases was observed. Computational analysis identified breast cancer biomarker Arid4b as a top target of miR-290-3p, which was confirmed by luciferase reporter assay. In conclusion, these results suggest germline genetic changes may reduce miR-290 expression, in turn increasing Arid4b expression, to create a predisposition towards breast cancer progression.

B21 The paralogous microRNA clusters miR-17-92 and miR-106-25 are specifically overexpressed in metastatic non-small cell lung carcinomas. Katey SS Enfield, Stephen Lam, Wan L. Lam. BC Cancer Research Centre, Vancouver, BC, Canada.

Background: Lung cancer is the cause of the most cancer-related deaths worldwide, with poor survival being largely attributed to late stage of disease at diagnosis and frequent metastasis. Understanding the mechanisms by which lung tumors metastasize could enable the development of anti-metastatic interventions or more specific therapeutics for metastatic disease. MicroRNAs (miRNAs) are major regulators of gene expression and control a wide range of cellular processes involved in metastasis, including apoptosis and cell cycle progression. Recently, increased expression of two paralogous miRNA clusters, *miR-17-92* and *miR-106b-25*, was tied to control of these functions through antagonizing transforming growth factor-β (TGFβ) signaling. A tumor

suppressive cytokine, TGFβ regulates cell cycle progression and apoptosis through activation of p21 and BIM, respectively. However, evidence suggests this regulation can be repressed by the overexpression of these microRNA clusters to promote tumor progression. To this end, we sought to determine whether or not expression of these clusters was increased in non-small cell lung cancer (NSCLC) cases positive for nodal or distant metastases compared to those with only locally invasive disease.

Methods: A panel of 41 non-metastatic NSCLCs and a panel of 28 NSCLCs with nodal or distant metastases were collected along with paired adjacent non-malignant tissues. Expression analysis of miRNAs was conducted in these specimens using Illumina GaXII small RNA sequencing technologies. Matched tumor and normal miRNA normalized read count comparisons were performed for each miRNA in the *miR-17-92* (*miR-17*, *miR-18a*, *miR-19a, miR-20a, miR-19b-1, miR-92a-1*) and *miR-106b-25* (*miR-106b, miR-93, miR-25*) clusters (Wilcoxon Signed-Rank test $p<0.05$). Only those miRNAs that were significantly overexpressed and displayed a minimum average expression fold change of 2 were further investigated.

Results: The significant overexpression of several miRNAs occurred specifically in the metastatic cohort, and included *miR-20a*, *miR-92a-1*, *miR-106b* and *miR-93*. *miR-18a* and *miR-19a* were significantly overexpressed in both tumor cohorts as compared to matched normal tissue; however, expression levels were substantially higher in the metastatic cohort and increased by 9.5 and 3 fold, respectively. *miR-17* and *miR-19b-1* were upregulated in both cohorts to a similar level, while expression of *miR-25* was not significantly altered.

Conclusion: miRNAs in the *miR-17-92* and *miR-106b-25* clusters, save *miR-25*, were significantly overexpressed in NSCLC cases positive for nodal or distant metastases. There was an overall trend of increasing involvement of miRNAs from these clusters moving from NSCLC cases without metastases to those with metastases, suggesting upregulation of these miRNAs is involved in the metastatic process.

B22 miR-34c is a putative tumor suppressor microRNA in Dicer-Pten double knockout high-grade serous carcinomas. Zhifeng Yu, Jaeyeon Kim, Chad Creighton, Shannon Hawkins, Martin Matthew Matzuk. Baylor College of Medicine, Houston, TX.

MicroRNAs (miRNAs) are short non-coding RNAs that could have large-scale biological effects by directing gene regulation through translational repression and degradation of multiple complementary target mRNAs. Like other regulatory molecules, altered miRNA expression has been suggested to be involved in the formation of many human diseases, including ovarian cancer. Our laboratory created a mouse model with high-grade serous carcinomas by conditionally deleting both *Dicer* (essential for miRNA biosynthesis) and *Pten* (a negative regulator of the PI3K pathway) in the female reproductive tract. Due to the fact that *Pten* knockout alone did not result in serous ovarian cancer, the significance of the impaired mature miRNA biosynthesis should be highly emphasized in this *Dicer-Pten* double knockout (DKO) high grade-serous carcinomas. To define the specific miRNAs in this DKO mouse model, we created cell lines derived from primary ovarian tumors in the *Dicer-Pten* DKO mice. Lack of *Dicer* makes the cell lines generated from these mice to be a valuable platform on functionally evaluating the significance of miRNAs in this model. We delivered control miRNA, *miR-31*, *miR-100*, *let-7b*, and *miR-34c* mimic to *Dicer-Pten* DKO cell lines by transient transfection, due to our previous studies of these miRNAs in human ovarian cancer or the abundance of these miRNAs in mouse normal fallopian tubes. We observed a growth inhibitory effect of *miR-34c* that is accompanied with cell cycle arrest at G1 phase and induction of apoptosis. *miR-34c* is a direct transcriptional target of p53 whose mutation is the most frequent in human ovarian cancers. Using quantitative real-time PCR, *miR-34c* levels were found to be extremely low in human serous adenocarcinomas compared with normal fallopian tube. Enforced expression of *miR-34c* in a human serous ovarian cancer cell line induced cell growth arrest further

indicating that our data on *miR-34c* in our mouse model could be translated to women.

B23 Selective inhibition of a long noncoding RNA (lncRNA), MALAT1 by antisense oligonucleotides results in significant antitumor effects in a variety of preclinical cancer models.. Jeff Hsu, Guobin He, Gourab Bhattacharjee, Tianyuan Zhou, Chris May, Xiaokun Xiao, Gene Hung, Brett P. Monia, A. Robert MacLeod, Youngsoo Kim. Isis Pharmaceuticals Inc., Carlsbad, CA.

An increasing body of evidence supports the notion that long noncoding RNAs (lncRNAs) play important roles in diseases including cancer. MALAT1 (also called NEAT2) was originally identified in tumors of highly metastatic non-small cell lung cancer (NSCLC), however, the functional role of MALAT1 in carcinogenesis has remained largely unknown. However, MALAT1 is highly expressed in various types of cancer and is implicated in the regulation of alternative splicing. To determine if MALAT1 constitutes an important driver of tumor growth in vivo, we developed potent antisense oligonucleotides (ASOs) that were able to achieve strong inhibition of MALAT1 in the tumor cells of a variety of preclinical models following systemic delivery. MALAT1 ASOs were well tolerated in rodents at doses leading to >95% inhibition of MALAT1 RNA in the liver. Inhibition of MALAT1 expression in tumor and tumor-associated stromal cells was determined by both q-RT-PCR using species-specific probe/primer sets and/or in situ 'viewRNA' technology, where target knockdown can be visualized on a cell by cell basis. Systemic administration of mouse MALAT1 ASOs resulted in a decrease in the numbers of polyps and proliferation index (BrdU (+)) in the small intestine of *Apc^{min}* mouse model of colon cancer and correlated well with MALAT1 inhibition in the polyps. Mouse MALAT1 ASOs were also effective in a DEN-induced HCC model, where ASO treatment reduced the target RNA ~ 90% in tumor cells with a concomitant decrease in tumor numbers, while control ASO had no effects on either measure. In addition, higher expression of MALAT1 in non-treated tumors compared to adjacent normal hepatocytes was also clearly visualized by the in situ 'view RNA' method. Furthermore, MALAT1 ASO significantly delayed tumor growth in C26 colon cancer and reduced tumor size in TRAMP mouse model of prostate cancer, where the target RNA was decreased by 80% in tumor cells. In a human NSCLC patient-derived xenograft model, both significant MALAT1 RNA reduction and a delay in tumor growth were achieved after MALAT1 ASO treatment. The effects of MALAT1 downregulation by ASO were not limited to the inhibition of tumor growth alone. MALAT1 ASO treatment not only inhibited the growth of primary tumors in the EBC-1 human NSCLC xenograft model, but also resulted in a decrease in lung metastasis as measured by micro CT scanning. Furthermore, cross-species MALAT1 ASOs greatly improved the survival of animals bearing Hep3B human hepatocellular carcinoma (HCC) tumor orthotopically (48.5 days with control ASO *vs* 88 days with MALAT1 ASO, p=0.005). Taken together, these results demonstrate previously undiscovered roles of MALAT1 as an important regulator in vivo tumor growth and metastasis and suggest that selective inhibition of MALAT1 by ASO could have therapeutic value for the cancer treatment.

B24 New views on microRNAs involvement in melanoma. Eyal Greenberg[1], Eran Eyal[2], Gideon Rechavi[2], Noam Shomron[1], Jacob Schachter[2], Gal Markel[2], Yael Nemlich[1], Steven Hajdu[2], Orit Itzhaki[2], Liat Edry[1], Oz Solomon[2], Ninette Amariglio[2], Michal J. Besser[2], Yona Keisari[1]. [1]Tel Aviv University, Tel Aviv, Israel, [2]Sheba Medical Center, Ramat Gan, Israel.

MicroRNAs (miRNAs) are small non-coding RNAs with regulatory roles, which are involved in a broad spectrum of physiological and pathological processes, including cancer. Most published data is focused on miRNAs that are differentially expressed between melanocytes and melanoma cells. These experiments lead to the discovery of a plurality of new miRNA-based mechanisms. However, this widely common approach fails to address at least two important issues: a) the effect of genomic mutations on target gene regulation by miRNAs; b) the

role of miRNAs in shaping the phenotypic heterogeneous nature of metastatic disease. Here we address these issues to provide new insights on the pathogenesis of melanoma and facilitate the development of innovative diagnostic and therapeutic implementations.

Melanoma cells acquire tens of thousands of genomic mutations, most of which are defined as "passengers" and few as "drivers". We analyzed a fully sequenced genome of melanoma tumor and found that >200 mutations hit the 3'UTR of genes, the main target area of miRNAs (out of 499 that hit transcribed genes). Three different computational algorithms (PITA, miRanda and our in-house tool, MirHB) concur and predict that these somatic mutations globally reduce the binding of miRNAs to the mutated 3'UTRs. This phenomenon is not genome-specific and proved to reflects the nature of the characteristic UV-induced mutation, C-to-T. Accordingly, mutation patterns in other malignancies, which are not primarily induced by UV-radiation, such as lung cancer and AML, do not yield similar predictions. This indicates that miRNA-based regulation can progressively perturb even with no apparent alterations in the expression of miRNAs. We define these mutations as "dormant drivers", as each mutation is expected to have a moderate effect, but the cumulative effect is significant.

We next identified miRNAs that regulate the aggressive phenotype of established melanoma cells. For that purpose, we tested two isogenic human melanoma cell lines (derived from the same patient) that dramatically differ in net proliferation, invasion, tube formation and tumorigenicity in vivo. Comparative screening revealed a large cohort of differentially expressed miRNAs. Molecular manipulation of highly ranked miRNAs (miR-31, -34a, -184, -185 and -204 and miR-17-5p) resulted in significant changes of the aggressive phenotype in vitro and in vivo. Remarkably, none of these miRNAs has been studied in cutaneous melanoma before. In conclusion, this study might lead to the development of novel lines of therapy.

B25 Characterization of a novel pseudogene expressed antisense RNA that regulates PTEN expression. Per Johnsson[1], Amanda Ackley[2], Dan Grandér[1], Kevin V. Morris[2]. [1]Karolinska Institutet, Stockholm, Sweden, [2]The Scripps Research Institute, La Jolla, CA.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR3) of the conference *Proceedings*.

B26 microRNA-181a regulates ovarian cancer progression and is associated with poor outcome in late-stage epithelial ovarian cancer. Aditya Parikh, Analisa DiFeo, Christine Lee, Alessia Baccarini, Valentin Kolev, Jamal Rahaman, Peter Dottino, Tamara Kalir, Brian D. Brown, Goutham Narla. Mount Sinai School of Medicine, New York, NY.

Epithelial ovarian cancer (EOC) is the fifth leading cause of cancer death in women and the most lethal gynecologic malignancy. The poor overall survival of this disease is due to both late stage diagnosis and the development of chemotherapy resistance. Although numerous studies have investigated the mechanisms of drug resistance, effective targets to overcome chemotherapy resistance and methods to predict an individual's response to a specific therapy are still lacking due to the variety and complexity of molecules and pathways involved in drug resistance. Deciphering the mechanisms that lead to chemotherapy resistance is an important challenge and ideally, a regulatory molecule, such as microRNAs (miRNAs), that can regulate the expression and function of multiple genes associated with a chemoresistant phenotype would be the ideal target for the development of therapeutics and/or biomarker for chemotherapeutic response. Here, we show that miR-181, a microRNA that is induced by MYCN and TGF-β and upregulated during differentiation, promotes epithelial-to-mesenchymal transition (EMT) and correlates with poor patient outcome in late-stage epithelial ovarian cancer (25 months vs. 66 months; p=0.002). Additionally, our results suggest that Smad7

is a direct and functional target of miR-181 and enforced expression of miR-181a alone was sufficient to activate TGF-β signaling. Furthermore, ectopic overexpression of miR-181a resulted in increased cellular survival, migration and in vivo tumor burden as well as drug resistance. In contrast, stable inhibition of miR-181 using a lentivirus-based sponge decoy vector inhibited TGF-β –dependent transcriptional responses and diminished cellular survival, migration and in vivo tumor dissemination. This data suggests that miR-181a represents a genetic determinant of cellular response to TGF-β through the direct regulation of the inhibitory Smad, Smad7, and introduces a novel biomarker and therapeutic target for late-stage ovarian cancer.

B27 Novel nanoparticle conjugated microRNA delivery reveals new tumor suppressors for neuroblastoma. Rajib Ghosh[1], Jayarathne Lalithya[1], Chen Zaowen[2], Hsu Danielle[2], Jason M. Shohet[2], Preethi H. Gunaratne[1]. [1]University of Houston, Houston, TX, [2]Texas Children's Cancer Center, Houston, TX.

High-risk neuroblastoma (NB) represents a major clinical challenge in pediatric oncology. A decade after the identification of MYCN as the key driver of NB, the five-year overall survival is less than 40% suggesting that signaling pathways downstream of MYCN need to be explored urgently. The future promise of microRNA-based therapeutics is based on targeting or replacement of these ~22 nucleotide RNAs that are commonly deregulated in many of the known diseases. The large size and polyanionic nature of naked miRNAs hamper cellular uptake. Viral delivery requires cloning, miRNA mimics/inhibitors need expensive modifications and both require electroporation or lipofection. We have developed and tested 35 unique formulations of amine functionalized gold nanoparticle (AuNP), unmodified miRNA (miR) and polyethylene glycol (PEG) conjugates. Using these conjugates we have tested two tumor suppressor candidates miR-31 (Creighton et al. 2010) and miR-1323 predicted to target sites in both MYCN and ALK (key oncogenes driving NB) in the neuroblastoma cell lines NGP and SY5Y. We found that cAuNPs10-miR1-PEG0.5 conjugates, which had the highest payload were non-toxic, taken up by 90% of cells in 20 minutes without the need for electroporation or lipofection. In addition they exhibited rapid endosomal escape and retained their specificity with respect to gene targets and phenotypic impact up to 5 days following delivery. miR-31-AuNP conjugates down-regulated predicted targets E2F2, STK40 and CEBPA and decreased proliferation of NGP and SY5Y revealing a new tumor suppressor microRNA for NB.

Surprisingly, miR-1323-AuNP conjugates significantly increased proliferation, clonigenicity and in vivo tumor growth of neuroblastoma lines SY5Y and CHLA255. As siRNAs to MYCN and ALK decrease viability of NB cell lines, this suggested that the oncogenic function of miR-1323 is likely to result from the repression of a tumor suppressor for NB. A search for putative tumor suppressive miR-1323 target genes uncovered Cbp/PAG1 as novel tumor suppressor for NB that is potently down-regulated by miR-1323. The transmembrane adaptor protein Cbp/PAG strongly suppresses proliferation driven by the Src family of kinases (SFKs) by sequestering them on lipid rafts of the cell membrane. Src inhibitors such as pyrazolo[3,4-d]pyrimidine derivatives, PP2, and Dasatinib have been demonstrated to substantially suppress proliferation and increase anti-tumor activity in vitro and in vivo mouse models of NB. Importantly we found a strong correlation of low Cbp/PAG1expression with a striking decrease in overall survival compared to patients carrying tumors with high levels of Cbp/PAG1 (overall survival $p < 0.001$). Our studies of microRNA function in neuroblastoma have uncovered Cbp/PAG1as a novel tumor suppressor and therapeutic tool for high risk neuroblastoma.

B28 microRNA-641 activates MAPK by targeting NF1 and cooperates with its host gene AKT2 in human cancer. Edward J. Richards, Marc Coppola, Jianping Guo, William Kong, Jin Q. Cheng. H. Lee Moffitt Cancer Center, Tampa, FL.

AKT2 is frequently up-regulated in human cancer. MiR-641 is an uncharacterized microRNA that locates at intron-1 of the AKT2 gene. We hypothesize that miR-641 and AKT2 are co-regulated, and cooperate to promote tumor development. Here we demonstrated that the transcription factors which induce AKT2 also increase miR-641 expression. Further, we identified miR-641 activation of the MAPK pathway by targeting tumor suppressor NF1, a GTPase-activating protein that inhibits RAS. Upon ectopic expression of miR-641, NF1 expression was reduced. As a result, pERK was elevated. Whereas knockdown of miR-641 exhibits opposite effects. In addition, depletion of miR-641 sensitizes tumor cells to cisplatin-induced apoptosis. Knockdown of miR-641 synergized with AKT2 inhibition to induce cell death. These data suggest that miR-641 functions as an oncomiR that co-regulates and cooperates with AKT2 in human cancer.

B29 A feedback regulatory loop between miR-125b and BRMS1 is linked to breast tumor progression. Delphine Allard[1], Magali Lacroix-Triki[2], Henri Roche[2], Fabrice André[1], Stephan Vagner[1], Gerard Maillot[2], Loubna Mhamdi[2], Sandra Pierredon[2], Florine Obrist[1], Aicha Goubar[1], Thomas Filleron[2], Margaux Roques[2], Véronique Scott[1]. [1]INSERM U981, Institut Gustave Roussy, Villejuif, France, [2]INSERM U563, Institut Claudius Regaud, Toulouse, France.

A plethora of studies showed altered microRNA (miRNA) expression in cancer. However, few reports investigated the role of miRNAs in the metastatic process. Here we used a mouse mammary tumor model (the 4T1 model), in which a set of four distinct tumor cell populations are able to complete distinct steps of metastasis when implanted into the mammary glands of BALB/c mice. MiRNA profiling by microarrays allowed us to show that each of the four tumor types is characterized by a specific cluster of regulated miRNAs.

More specifically, we found that miR-125b, which is overexpressed in the set of murine tumors that are capable of generating metastasis, increases cell invasion in vitro and formation of metastasis in vivo. Furthermore, miR-125b expression is significantly associated to metastatic outcome in two independent series of human breast tumors. In addition, we identified several miR-125b target genes, including *BRMS1* (Breast Metastasis suppressor 1) by immunoprecipitation of Argonaute protein-containing RNA-induced silencing complexes (RISCs) followed by microarray analysis. Importantly, siRNA-mediated depletion of BRMS1 limits the effects of miR-125b on invasion in vitro. Since we found that BRMS1 also represses miR-125b, we demonstrate the existence of a BRMS1/miR-125b regulatory feedback loop linked to tumor cell invasion and metastasis.

B30 Exogenous miR-128 enforced HNSCC inhibits proliferation. Belinda Hauser[1], Yubin Hao, Joseph Califano[2], Xinbin Gu[1]. [1]Howard University, Washington, DC; [2]Johns Hopkins University, Baltimore, MD.

Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous disease with complex molecular abnormalities. There are more than 65,000 Americans diagnosed with head and neck cancer and 25,000 die from this disease. microRNAs are 22~nucleotide-long endogenously expressed, highly conserved noncoding RNAs with important regulatory functions in proliferation, apoptosis, and metastasis. miR-128 a brain-enriched microRNA most commonly in gliomas that have shown to function as a tumor suppressor which decreases cell proliferation and cell invasiveness in prostate cancer. miR-128 has been shown to target BMI-1 in squamous cell carcinoma; however we believe miR-128 functions by targeting multiple targets in HNSCC.

Methods: We established HNSCC cell lines that stably expressed individual members of the miR-128 using a lentiviral delivery system. The levels of miR-128 and their targeted

proteins were analyzed by qRT-PCR and Western blot. miR-128 affinity was elucidated by MTT, colony formation, flow cytometry, and a tumor xenograft model.

Results: Enforced transfection of miR-128 in HNSCC (JHU-22) expression was stable in vitro and in vivo. We identified five specific targets of miR-128 (BMI-1, BAG2, BAX, H3f3b, and Paip2). BMI-1 and H3f3b were shown to be a more dominant targeted of miR-128. Overexpression of miR-128, lead to the down regulation of these targets including decrease cell viability (30%), decrease of proliferation (55%), and significantly reduced solid tumor formation in tumor xenografts models.

Conclusions: miR-128 exhibits anti-proliferative effects and phenotypic alteration in HNSCC by targeting multiple predicted conserved targets activity function in apoptosis, cell cycle, transcription, and translation. Therefore, screening for differentially expressed targets of miR-128 help elucidate the possible mechanisms underlining the function of miR-128.Our results indicate that miR-128 has the potential to serve as a therapeutic approach.

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