UC DAVIS
COMPREHENSIVE CANCER CENTER

18th Annual Cancer Research Symposium

September 27–28, 2012

Cancer Center Auditorium
4501 X Street, Sacramento

Courtyard by Marriott Hotel
4422 Y Street, Sacramento

NCI·CCC
A Comprehensive Cancer Center Designated by the National Cancer Institute

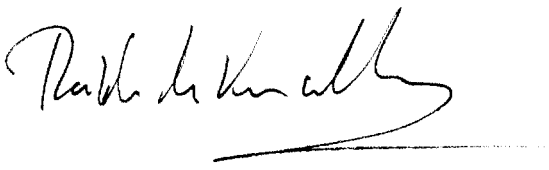
Can these mice explain how obesity impacts cancer therapeutics?

From the Director

As we kick off the 18th annual symposium, we can add another chapter to our history and development of a world class center. In 1991, at our inception, we were one of 6,500 buildings in the United States that had the label "Cancer Center" on them. In 2002, we achieved NCI designation and with it, we became one in sixty-some Cancer Centers in the country with this designation. Since then, we have twice been re-designated, and in March of this year we became the 41st Comprehensive Cancer Center in the country. Our challenge over the next five years is to ensure that our growth continues and even more importantly, that our impact on the whole of the Cancer Process increases.

As we continue to build the next generation of Cancer Center Investigators and Leaders, the importance of increasing the team science efforts of our present members is becoming more important. Our mission will be to maintain Comprehensive status, build and develop collaborations internally and externally, and develop more physician scientists. Over the past 18 years, our annual Symposium has been the venue where our ideas and collaborations come together to inspire future opportunities.

Thank you for being a part of the 2012 Cancer Center Symposium.
All the best,



Ralph W. de Vere White, M.D.
Director, UC Davis Cancer Center
Associate Dean for Cancer Programs
Codman-Radke Chair in Cancer Research
Distinguished Professor, Department of Urology

SYMPOSIUM COMMITTEE MEMBERS

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Vice Chair Research
Professor Department of Dermatology
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SPECIAL THANKS

For over two decades of amazing Physicians, Scientists, Staff and Volunteers who have helped build the Cancer center from opening day in 1991 to our newly designated Comprehensive status. Without your team efforts we would not be here today.

INDEX

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CANCER CENTER SYMPOSIUM 2012

Thursday, September 27, 2012

SESSION I. **APPROACHES TO HORMONE-FUELED TUMORS**
Chair: Colleen Sweeney, PhD

Time	Discussion	Presenter	Location
8:20 am – 8:30 am	Introduction	Ralph de Vere White, M.D.	Cancer Center Auditorium
8:30 am – 9:30 am	**Neuroendocrine Influences on Cancer Metastasis**	**Anil Snood, M.D. M.D. Anderson**	
9:30 am – 9:50 am	Impact of Psychosocial Stress in a Transgenic mouse model of ErbB2-positive breast cancer	Colleen Sweeney, Ph.D.	
9:50 am – 10:10 am	SOST Inhibits Prostate Cancer Invasion	Bryan Hudson, Ph.D.	
10:10 am – 10:20 am	Break	All	
10:20 am – 10:40 am	Epigenetic regulators in driving chemoresistance and endocrine therapy resistance	Hong-Wu Chen, Ph.D.	
10:40 am – 11:00 am	Development and preliminary Evaluation of Serum Glycan Biomarker Panels for Ovarian Cancer	Kyoungmi Kim, Ph.D.	
11:00 am – 11:20 am	Increasing Mammography Screening Among American Indian Women: Addressing the Cultural Barriers; Progress Report on the Athena Health Network Project: Comparative Effectiveness and Survivorship CCRT	Marlene von Friederichs-Fitzwater, Ph.D.	
11:20 am – 12:50 pm	Poster Session and Lunch	All	Marriott Ballroom

SESSION II. **TRANSLATIONAL RESEARCH**
Chair: Hsing-Jien Kung, PhD

Time	Discussion	Presenter	Location
1:00 pm – 2:00 pm	**Genomics Strategies in Oncogene Discovery**	**Edison Liu, M.D. C.E.O Jackson Laboratories**	Cancer Center Auditorium
2:00 pm – 2:20 pm	Targeting Arginine Addiction of Prostate Cancer	Hsing-Jien Kung, Ph.D.	
2:20 pm – 2:40 pm	Presentation of the David R. Gandara Lecture on Developmental Therapeutics Assessment of Inter- and Intra-Patient Tumor Heterogeneity through	David Gandara, M.D.	

	a Novel Research Platform integrating data from Genetically Engineered Mice, Patient Derived Xenografts and Clinical Trials (iGXT)		
2:40 pm – 3:00 pm	ER+ Mouse Mammary Carcinoma, Hormonal Independence, EMT and Tp53 mutation	Alexander “Sandy” Borowsky, M.D.	
3:00 pm – 3:20 pm	Animal models of human prostate cancer: The Consensus Report of the New York Meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee	Robert Cardiff, M.D., Ph.D.	
3:20 pm – 3:40 pm	Bone Metabolism Biomarkers in Castration Resistant Prostate Cancer (CRPC) Patients with Skeletal Metastases: Results from SWOG 0421	Primo “Lucky” Lara, M.D.	
3:40 pm – 4:00 pm	The Role of MXD3 in Human Precursor B Cell Acute Lymphoblastic Leukemia	Noriko Satake, M.D. Gustavo Barisone, Ph.D.	
4:00 pm – 4:20 pm	Overcoming Resistance to Tyrosine Kinase and Androgen Receptor Targeted Therapies in Castration-Resistant Prostate Cancer	Christopher Evans, M.D.	
Symposium Recess			

Friday, September 28, 2012

SESSION III. **DEVELOPMENTS IN NUTRITION AND CANCER**
Chair: Ralph de Vere White, M.D.

Time	Discussion	Presenter	Location
9:00 am – 11:00 am	Poster session and Continental Breakfast	All	Marriott Ballroom
11:00 am – 11:30 am	Towards Depersonalized Medicine	Richard Levenson, M.D.	Cancer Center Auditorium
11:30 am – 12:00 pm	Milk, the Microbiota and Cancer: What can Evolution teach us?	Bruce German, Ph.D.	
12:00 pm – 12:30 pm	Box Lunch	All	
12:30 pm – 12:45 pm	Poster Awards	Ralph de Vere White, M.D.	
12:45 pm – 1:05 pm	Four Horsemen: Age, Obesity, Inflammation, and Microphages	William Murphy, Ph.D.	
1:05 pm – 1:25 pm	Vitamin A and Liver Health	Yu-Jiu Yvonne Wan, Ph.D.	
1:25 pm – 1:45 pm	Diet, Immunity and Inflammation	Charles Stephensen, Ph.D.	
1:45 pm – 2:05 pm	Bringing Synbiotic Interventions in Cancer to Practice: from bench to bedside	Jennifer Smilowitz, Ph.D.	
Symposium Adjourn			

ORAL PRESENTATIONS

Cancer Center Auditorium

4501 X Street, Sacramento

KEYNOTE SPEAKER BIOGRAPHICAL INFORMATION

ANIL SOOD, M.D.

Director of the Blanton-Davis Ovarian Cancer Research Program M.D. Anderson Cancer Center

Dr. Sood is Professor and Director of the Blanton-Davis Ovarian Cancer Research Program in the Departments of Gynecologic Oncology and Cancer Biology at the M. D. Anderson Cancer Center. His research is focused in three main areas: 1) development of new strategies for systemic *in vivo* siRNA delivery using biocompatible nanoparticles; 2) effect of neuroendocrine stress hormones on ovarian cancer growth and progression; and 3) development of novel anti-vascular therapeutic approaches. Dr. Sood has published numerous peer-reviewed articles and has authored and co-authored several book chapters. Dr. Sood has received major recognition for his research accomplishments including the Gynecologic Cancer Foundation/Margaret Greenfield/Carmel Cohen, M.D. "Excellence in Ovarian Cancer Research Award".

Research Interests

Dr. Sood's research is focused in three main areas: 1) Effects of neuroendocrine pathways on ovarian cancer growth and progression. The adverse effects of stress pathways on the immune system have been known for a long time. My laboratory made the novel observation that stress hormones can have direct effects on ovarian cancer cells via beta-adrenergic receptors and promote angiogenesis, tumor growth and metastasis. Ongoing work is focused on dissecting the molecular and biological processes involved in mediating the effects of stress biology on tumor microenvironment. 2) Development of novel anti-vascular therapeutic approaches. Our ongoing studies in this area are focused on understanding the functional significance of key tyrosine kinases such as EphA2 and focal adhesion kinase in tumor angiogenesis. In addition, we are examining the relevance of perivascular cells in protecting tumor-associated endothelial cells. Based on these findings, we have developed novel strategies for targeting both endothelial cells and pericytes in ovarian cancers. 3) Development of new strategies for systemic in vivo siRNA delivery. The use of siRNA as a method of gene silencing has rapidly become a powerful tool for determining protein function and gene discovery. The extraordinary sequence specificity of siRNA makes it an attractive tool for cancer therapy. We are utilizing nanovectors for highly efficient in vivo delivery of siRNA with the hope of translating this work for clinical applications in the near future.

KEYNOTE SPEAKER BIOGRAPHICAL INFORMATION

EDISON LIU, M.D.
President and Chief Executive Officer, Jackson Laboratory

Edison T. Liu is an international leader in cancer biology, genomics, human genetics and molecular epidemiology. He joined The Jackson Laboratory as President and CEO in January 2012 after an extensive international search.

Dr. Liu founded the Genome Institute of Singapore in 2001, building it in less than 10 years from a staff of three to a major research institute of 27 laboratory groups and a staff of 270. From 1996 to 2001, he was the scientific director of the National Cancer Institute's Division of Clinical Sciences in Bethesda, Md. He is serving his second term as the elected president of the international Human Genome Organization (HUGO). From 1987 to 1996 he held various faculty positions at the University of North Carolina at Chapel Hill in the departments of medicine, epidemiology, biochemistry and biophysics, and in the curriculum in genetics. He also held leadership positions at UNC including director of the Lineberger Comprehensive Cancer Center's Specialized Program of Research Excellence in Breast Cancer and the Laboratory of Molecular Epidemiology in the School of Public Health.

Dr. Liu obtained his B.S. in chemistry and psychology, as well as his M.D., at Stanford University. He served his internship and residency at Washington University's Barnes Hospital in St. Louis, followed by an oncology fellowship at Stanford. From 1982 to 1987 he was at the University of California, San Francisco, first in a hematology fellowship at Moffitt Hospital and then as a postdoctoral fellow in the laboratory of Nobel Laureate J. Michael Bishop, while also serving as an instructor in the School of Medicine.

Dr. Liu is married and has three children. In his spare time, he enjoys jazz piano and composition, and writes for the lay public in newspapers and magazines about science, medicine and society.

ABSTRACTS OF ORAL PRESENTATIONS: THURSDAY

SESSION I. APPROACHES TO HORMONE-FUELED TUMORS

KEYNOTE LECTURE: NEUROENDOCRINE INFLUENCES ON CANCER METASTASIS

Anil Sood, M.D.

Director of the Blanton-Davis Ovarian Cancer Research Program, M.D. Anderson Cancer Center

Adrenergic signaling has been found to regulate multiple cellular processes that contribute to the initiation and progression of cancer, including inflammation, angiogenesis, apoptosis/anoikis, cell motility and trafficking, activation of tumor-associated viruses, DNA damage repair, cellular immune response, and epithelial–mesenchymal transition. In several experimental cancer models, activation of the sympathetic nervous system promotes the metastasis of solid epithelial tumors and the dissemination of hematopoietic malignancies via β-adrenoreceptor–mediated activation of protein kinase A and exchange protein activated by adenylyl cyclase signaling pathways. Within the tumor microenvironment, β -adrenergic receptors on tumor and stromal cells are activated by catecholamines from local sympathetic nerve fibers (norepinephrine) and circulating blood (epinephrine). Tumor-associated macrophages are emerging as key targets of β-adrenergic regulation in several cancer contexts. Sympathetic nervous system regulation of cancer cell biology and the tumor microenvironment has clarified the molecular basis for long-suspected relationships between stress and cancer progression, and now suggests a highly leveraged target for therapeutic intervention. Epidemiologic studies have linked the use of β-blockers to reduced rates of progression for several solid tumors, and preclinical pharmacologic and biomarker studies are now laying the groundwork for translation of novel adjuvant methods to existing therapeutic strategies in clinical oncology.

IMPACT OF PSYCHOSOCIAL STRESS IN A TRANSGENIC MOUSE MODEL OF ERBB2-POSITIVE BREAST CANCER

Colleen Sweeney, Hanine Rafidi, Elizabeth Takahashi, Jessica Wald and Brian Trainor

Epidemiologic as well as pre-clinical studies strongly suggest that psychosocial stress contributes to tumor growth and progression although the mechanisms involved are not fully understood, limiting clinical translation. In breast cancer, stress-related psychosocial factors are associated with decreased survival, emphasizing the need for a better understanding of how stress intersects with tumor biology in this disease. Psychosocial stress precipitates activation of the autonomic nervous system (ANS) and the hypothalamic pituitary adrenal (HPA) axis, prompting the release of catecholamines and glucocorticoids. These factors may impact tumor biology directly through effects on tumor cells or indirectly, through effects on the tumor microenvironment. We used restraint stress to model the effects of psychosocial stress on ErbB2-dependent mammary tumorigenesis. Approximately 20% of human breast cancers are ErbB2-positive and this model faithfully recapitulates the hallmarks of ErbB2-positive disease. Mice in which ErbB2 transgene expression was selectively induced in the mammary gland were randomly assigned to restraint stress or control. We find that chronic stress has no effect on the kinetics of tumor appearance (tumor latency) but does significantly increase tumor burden. Interestingly, inhibition of acute stress responses by pre-treatment with propranolol (-adrenergic receptor blocker) or metyrapone (blocks corticosterone synthesis) did not reverse the effects of stress on tumor burden. We hypothesize that chronic stress leads to a readjustment in the basal or “tonic” output of the ANS and HPA axis which in turn drives stress effects on tumor burden. Indeed, basal corticosterone levels were positively correlated with tumor number. Our data suggest that long-term rather than acute inhibition of stress-related pathways may be necessary to limit their tumor-promoting effects.

SOST INHIBITS PROSTATE CANCER INVASION

Bryan D. Hudson[1], Nick Hum[1], Cindy Thomas[1] and Gabriela G. Loots[1]

[1]Biosciences and Biotechnology Division, LLNL, Livermore, CA.

In addition to its role in bone development, metabolism, and repair, the WNT signaling pathway is also implicated in cancer oncogenesis. Several inhibitors of WNT signaling, including dickkopf homolog 1 (DKK1), Wnt inhibitory factor 1 (WIF1), and secreted frizzled protein (sFRP), have been shown to be involved in prostate cancer (PC) metastasis; however the role of sclerostin (SOST) has not yet been explored. We examined human PC cells (PC3 and C4-2Bm) and healthy human prostate epithelial cells (HPrEC) to investigate the role of SOST in PC proliferation and invasion through the addition of recombinant proteins (rhSOST and rhDKK1) and in co-cultures with primary osteoblasts (OBs) isolated from mice lacking either the WNT co-receptor *Lrp5* (*Lrp5 KO*) or the WNT inhibitor *Sost* (*Sost KO*). We also conducted a whole-genome survey in PC3 to identify molecular changes as a function of PC3-OB co-culture. Here we report that rhSOST dramatically inhibits the invasive properties of both PC3 and C4-2Bm cells, without effecting cell proliferation or viability. In contrast, we found that rhDKK1 significantly increases invasion. Our data also shows that PC3 and C4-2Bm cells co-cultured with OBs derived from *Sost KO* mice exhibit increased invasion; an effect reversed in co-cultures with *Lrp5 KO* derived OBs. Gene expression analysis showed that major factors known to be involved in invasion, such as MMP2, are up-regulated in PC3 cells co-cultured with *Sost KO* OBs and down-regulated in *Lrp5 KO*, further implicating the direct involvement of WNT signaling in matrix degradation and cancer invasion. These results were further examined *in vivo,* following intrafemoral injections of PC3 cells in 8-week old SCID mice. Consistent with the *in vitro* data, we found intravenous injections of SOST decreased osteolytic growth of PC relative to sham controls. These findings support the hypothesis that the WNT signaling pathway is a critical component of prostate cancer metastasis to bone. In addition, we show that PC invasion is strongly reliant on SOST availability, where loss of SOST and hyperactive WNT activity in osteoblasts and bone tissue increases the ability of PC to invade and form tumors.

Disclosures: *None.*
This work conducted under the auspices of the USDOE by LLNL (DE-AC52-07NA27344).

EPIGENETIC REGULATORS IN DRIVING CHEMORESISTANCE AND ENDOCRINE THERAPY RESISTANCE

Hongwu Chen

UC Davis Cancer Center, Basic Sciences; Department of Biochemistry & Molecular Medicine

Resistance to chemotherapy or endocrine therapy still presents a major challenge in the management of breast cancer. Multiple cellular pathways are implicated in the resistance development. Identification of key mediators of the resistance that integrate the different pathways can be valuable to effective prediction of tumor response and offering new targeting opportunities. We recently identified an epigenetic reader protein ANCCA/ATAD2 as a key regulator of cancer cell proliferation and survival and revealed its expression association with poor outcomes. Our further studies demonstrate that ANCCA is induced by chemotherapeutic agents such as adriamycin and carboplatin for the expression and function of DNA damage repair proteins including BRCA1, Chk1 and members of the Rad51 family. High levels of ANCCA mediate DNA damage response, cell cycle checkpoints and DNA damage repair. Its depletion strongly sensitizes cancer cells to killing by the chemo-drugs. Interestingly, ANCCA and its target histone methylase NSD2 are also potent drivers of ER-positive cancer cell and tumor resistance to endocrine therapies such as estrogen deprivation and tamoxifen treatment. One major underlying mechanism is their aberrant function to activate the EGFR/HER2 and Akt pathways. Together, these findings underscore the importance of key epigenetic regulators in cancer therapy resistance and as novel therapeutic targets.

DEVELOPMENT AND PRELIMINARY EVALUATION OF SERUM GLYCAN BIOMARKER PANELS FOR OVARIAN CANCER

Kyoungmi Kim, PhD, Associate Professor, Division of Biostatistics, University of California Davis School of Medicine

Diagnostic biomarkers are used to make predictions on patients suspected of having ovarian cancer. Most of the ovarian tumor protein biomarkers (CA125, HE4, transthyretin, CA15.3, and CA72.4) that are FDA approved in the diagnosis of ovarian cancer but not approved yet for ovarian cancer screening are glycosylated. The recent advances in glycomics techniques allow us a global search for novel glycan biomarkers. An interdisciplinary research team composed of clinicians, biomedical researchers, and biostatisticians has been established at UC Davis Cancer Center to examine glycans adducted to proteins in serum to discover those that are differentially present in patients with ovarian cancer compared to non-cancer controls using global glycomic analysis. In this presentation, we describe our ongoing local effort towards the development of a multiplex glycan biomarker panel which may be useful for an early diagnostic test as well as to elucidate new targets for therapy.

INCREASING MAMMOGRAPHY SCREENING AMONG AMERICAN INDIAN WOMEN: ADDRESSING THE CULTURAL BARRIERS

Marlene von Friederichs-Fitzwater, Ph.D.

The current three-year grant builds on the successful results of the California Breast Cancer Research Program funded 18-month pilot project, using the culturally sensitive Mother's Wisdom Breast Health Program intervention to increase mammography screening rates among American Indian/Alaska Native (AIAN) women by at least 18% from an average of 42% to 60% or higher. At the end of two years, three of the five partnering tribal health clinics report mammography screening rates averaging 51.3%, an increase of 30% over 2009. The other two clinics had personnel changes , but did have small increases. Results from this study at the end of the three-year period will demonstrate the value and sustainability of the Mother's Wisdom Breast Health Program in increasing mammography screening rates among American Indian women.

PROGRESS REPORT ON THE *ATHENA* HEALTH NETWORK PROJECT: COMPARATIVE EFFECTIVENESS AND SURVIVORSHIP CCRT

Marlene von Friederichs-Fitzwater, Ph.D.

Survivorship care, especially with regard to coordination of post-treatment care, is a national priority and is well described in the 2006 Institute of Medicine (IOM) report, *From Cancer Patient to Cancer Survivor: Lost in Transition*. This report includes the results of a qualitative study done to examine provider perceptions and expectations of post-treatment breast cancer across five UC Network sites: UC San Francisco, UC Irvine, UC Los Angeles, UC San Diego and UC Davis. Semi-structured telephone and in-person interviews were conducted with oncology specialists and primary care providers. Results identified an unprompted major theme of "the need for care coordination" and 69% of the respondents stated that implementing survivorship care programs was a way to improve care delivery. A survivor study is underway at all five sites to gather data on patient expectations and patterns of post-treatment breast cancer care. Results will be used to develop survivorship care programs.

SESSION II. TRANSLATIONAL RESEARCH

KEYNOTE LECTURE: GENOMICS STRATEGIES IN ONCOGENE DISCOVERY

Edison Liu, M.D.

President and Chief Executive Officer, Jackson Laboratory

Genomic medicine involves the provision of medical care that uses the power of genomic knowledge and technologies to resolve complex problems. The fundamental difference between this and older strategies in medicine research is the comprehensiveness and the precision of the analyses afforded by new genomic technologies such as in sequencing, cloning, and genotyping. The new challenge will be the assembly and management of this high volume of data with dimensional complexity. Genomic medicine therefore means computational and systems medicine as well. Operationally, systems biology requires the digitalization of biological output, the computational power to analyze comprehensive and massive datasets, and the capacity to integrate heterogeneous data into a usable knowledge format. There is no field more suitable for genomic strategies in clinical care than cancer.

We will describe how genomic approaches are changing our understanding of cancer, as a model system. We employ a strategy of extracting biological information from genomic data and to reconstruct systems maps of critical regulatory networks. This integrative approach permits modeling of complex interactions and allows us to pinpoint novel oncogenes and tumor suppressor genes. Coupled with the dramatic expansion of disease gene discovery in population studies, we now find that rather than a few genes, hundreds of genes may be involved in the genesis of a single complex disease. Harnessing complexity will be our next great challenge.

TARGETING ARGININE-ADDICT OF PROSTATE CANCER

Hsing-Jien Kung, Austin Changou, Randie Kim, Richard Bold and Frank Chuang

UC Davis Cancer Center, UCDMC, Sacramento, CA, USA.

There is considerable evidence that tumor and normal cells differ in their metabolic requirements. The most prominent examples are the addiction of tumor cells to glucose (i.e., Warburg effect) and to glutamine. Therapeutics based on selective targeting of these metabolic pathways is under intensive investigations.. In addition to glutamine, the differential requirement of other amino acids by tumor cells also exist and has been exploited to developing amino-acid depletion therapy. The choices, however are limited, as most of the amino acids are essential for normal cellular physiology, and only eight amino acids including arginine are considered semi-essential or non-essential.

Although amino-acid deprivation strategy has not been clinically used in prostate cancer treatment, androgen deprivation and anti-androgen therapy is the standard of care for patients with biochemical recurrence (i.e., PSA rising) after surgery or radiation . The recent advent of potent small-molecule inhibitors which target androgen binding (MDV3100) or androgen synthesis (Abiraterone) has improved the treatment prospects. Nevertheless, a third of patients treated with the new regimens still develop drug resistance, leading to castration-resistant prostate cancers (CRPC). At this juncture, the only treatment option is Taxol, which is generally toxic and prolongs the survival by a few months. Thus, there is an unmet need to develop effective adjunctive therapy or treatment strategy that is independent of androgen/AR axis. Recently, we reported that irrespective of AR status, prostate cancer cells selectively and epigenetically suppress the expression of ASS (arginine succinosythethase), a rate-limiting enzyme for intracellular arginine synthesis. Analysis of over 100 PC specimens showed the complete absence of ASS expression, whereas some normal prostate epitheial cells express ASS. As a result, PC cells, but not normal counterparts become “auxotroph” for and addicted to external arginine. Thus, arginine-deprivation should selectively “starve” the PC cells to death. Indeed, in recent publications, we showed that depletion of external arginine by arginine deiminase (ADI) effectively induces cell death of CRPC cell lines, but not normal prostate epithelial cells *in vitro* and *in vivo*. In addition, we reported that ADI synergizes with Taxol in preclinical xenograft model. Based on this finding, a phaseI/II clinical trial is underway at UCD. Intriguingly, we found that ADI killing of cancer cells is associated with agressive autophagy and appears to

be caspase independent. At early phase, autophagy is protective and prolongs the survival of treated cells. Using live imaging, molecular and genetic profiling, we have now characterized in details the arginine-deprived cells undergoing apoptosis. The starved cells showed significant epigenetic reprogramming, excessive autophagy and most remarkably, nuclear rupture. The possible mechanism(s) and its implication will be discussed.

ASSESSMENT OF INTER- AND INTRA-PATIENT TUMOR HETEROGENEITY THROUGH A NOVEL RESEARCH PLATFORM INTEGRATING DATA FROM GENETICALLY ENGINEERED MICE, PATIENT DERIVED XENOGRAFTS AND CLINICAL TRIALS (IGXT)

D.R. Gandara[1], T.A. Van Dyke[2], Z. Weaver Ohler[2], T. Li[1], P.N. Lara[1], P. Mack[1], D. T. Cooke[1], K. Yoneda[1], Suzanne Miyamoto[1], R. Gandour-Edwards[1], Stephanie H. Astrow[3], R. deVere White[1], N. Goodwin[5]

[1]UC Davis Comprehensive Cancer Center (UCDCCC) Sacramento, CA, [2]National Cancer Institute Frederick, MD, [3]Response Genetics, Inc. Los Angeles, CA, [4]The Jackson Laboratory Sacramento, CA

Background: Previously described preclinical models have proven suboptimal for directing clinical application of new anti-cancer therapies. Importantly, such models have failed to account for both inter-patient and intra-patient tumor heterogeneity intrinsic to human cancers. Here we describe an integrated research platform (iGXT) engaging core resources at The Jackson Laboratory (JAX-WEST), the NCI Center for Advanced Preclinical Research (CAPR) and clinical trials and laboratory resources based at the UCDCC. Pilot studies using this strategy are focusing on non-small cell lung cancer (NSCLC) due to molecular targets of interest, such as epidermal growth factor receptor (EGFR), heterogeneity in tumor biology and complexity of cancer signaling pathways.

Methods: In this pilot project, a pathway-specific approach to evaluating EGFR-related tumor biology and EGFR-directed drugs of interest is ongoing, integrating data from NCI CAPR in genetically engineered mice (GEMs) bearing tumors with defined EGFR-related characteristics, together with in vitro models at UC Davis, while NSCLC patients and JAX Nod Scid Gamma (NSG) mice with patient-derived xenografts (PDXs) from patients at UCDCCC. Patient tumors, GEMs and PDXs, pre- and post-therapy, are assessed by genome-wide technologies, integrated with data from CAPR, and extrapolated back to individual pt outcomes to gain insight into mechanisms of drug resistance and how to overcome them.

Results: To date, over 500 cancer patient tumors of various tumor types have been xenotransplanted into NSG mice, including ~75 NSCLC. In this pilot project, NSCLC PDXs show excellent histopathologic, mutational and gene expression fidelity, including molecular correlation, including mutation status for KRAS, EGFR and gene expression levels for a variety of genes of interest. A panel of EGFR-mutated NSCLC PDXs has been developed, highly annotated for clinical attributes and molecular characteristics. Pilot studies at CAPR in GEMs with EGFR mutant (Tet-op-EGFR L858R +/- T790M) tumors demonstrate efficacy of MK2206 +/- erlotinib and BIBW2992. Complementary in vitro studies at UCD with the c-MET inhibitor PHA-665752 or MK2206 confirm the ability of MK2206 to overcome c-MET-related resistance to erlotinib. Simultaneously, an ongoing erlotinib-MK2206 clinical trial with associated PDXs is designed to evaluate mechanisms of resistance to erlotinib and overcome them. In addition, studies evaluating a second generation EGFR TKI, afatinib, plus the EGFR-directed monoclonal antibody cetuximab, are ongoing. Demographics of host pts, histopatholgic features, molecular profiles and initial treatment results in PDXs and patients will be presented.

Conclusion: This EGFR-related pilot project supports the feasibility of systematically integrating data derived from iGXT models in order to optimize drug development and treatment strategies to address drug resistance mechanisms. Findings from this platform are likely to advance understanding of differences in inter- and intra-patient tumor biology and hasten the transition to personalized cancer therapy. Although this initial pilot project has focused on NSCLC and the EGFR pathway, we expect that this approach can be extrapolated to other tumor types and other molecular pathways.

ER+ MOUSE MAMMARY CARCINOMA, HORMONAL INDEPENDENCE, EMT, AND TP53 MUTATION.

A.D. Borowsky, S.R. Chan, R. D.Schreiber, R.D. Cardiff, N.E. Hubbard, R. J. Hovey, Q. J. Chen

Background: The 129S6/SvEv (Stat1-/-) mouse develops mammary carcinomas with a consistent estrogen receptor positive(ER+) phenotype. By definition, therefore, Stat1 is a functional tumor suppressor in the mammary gland. The consistent ER+ tumors occur late (>70wks) and with an incomplete penetrance suggesting that susceptibility in a population of mammary epithelial cells uniquely susceptible to the ER+ luminal type carcinomas requires additional factors. In this study we have begun to characterize the developmental heterogeneity of this mouse strain, and compared a series of tumors. We have established a series of cell lines from these mammary tumors, and one of these lines "SSM2-CA" retains the ER+ phenotype in culture and in syngeneic transplantations. We have tested the hormonal dependence and mechanisms of independence in this model.

Methods: 129SvEv(Stat1-/-) mice were obtained from Taconic Laboratories and used for transplantation and aging tumorigenesis studies. Syngeneic 129SvEv (wild type) were used as transplant recipients. Developmental phenotypes were assessed using a combination of cross transplantations of mammary gland and bone marrow to separately assess the effects of intrinsic epithelial biology, host tissue, and bone marrow/immune cell effects. Syngeneic transplantable tumor cell lines were used for cohort evaluation of hormonal effects with oophorectomy or mock procedure as well as exogenous administration of combinations of estrogen, progesterone, and/or prolactin. Tumor growth was monitored with caliper measurements, and timed sacrifice with histologic examination and immunohistochemistry were performed.

Results: Oophorectomy resulted in a plateau in growth rate followed by continued tumor growth. Histopathology shows a rapid wave of necrosis and apoptosis . Residual viable cells do not initially show a shift in cellular phenotype, but the resistant tumors that regrow after the initial cell death have a spindle cell morphology accompanied by increased basal keratins and vimentin, and loss of ER expression. Tp53 IHC staining (indicative of mutation induced protein stability) shows rare foci of positivity in controls and early timepoint post-oophorectomy , but at a high frequency in later resistant tumors.

Conclusions: These data show that estrogen independent growth of ER positive luminal carcinoma is associated with Tp53 mutation and a phenotype switch/redifferentiation of luminal cells to a basal/epithelial-mesenchymal transition phenotype. Specific blockers of EMT combined with anti-hormonal therapy may mitigate this mechanism of hormonal independence.

ANIMAL MODELS OF HUMAN PROSTATE CANCER: THE CONSENSUS REPORT OF THE NEW YORK MEETING OF THE MOUSE MODELS OF HUMAN CANCER CONSORTIUM PROSTATE PATHOLOGY COMMITTEE

Robert D. Cardiff[1], Alexander D. Borowsky[1], Michael Ittmann[2]

[1]UC Davis and [2]Baylor College

The National Cancer Institute's Prostate Cancer Steering Committee of the Mouse Models of Human Cancers Consortium (MMHCC) convened a panel of human and veterinary pathologists with expertise in PrCa (Prostate Cancer) to review the current state of the art in animal models of PrCa. A similar panel was convened 11 years ago resulting in the consensus report which defined the underlying principles and techniques for the pathological analysis of lesions in GEM PrCa models and provided detailed definitions for prostatic lesions in GEM. In the past 10 years, numerous new models have been developed and the scientific community has gained extensive experience in analyzing these models, necessitating a progress report. The new models were selected by the MMHCC PrCa Steering Committee. Blocks and/or slides were obtained from the investigators, slides were digitized and uploaded to the UCD Mouse Mutant Pathology Lab Image Archive database as Whole Slide Images (WSI). The panel of experts reviewed the slides and conducted pre-reviews over the internet and met for detailed discussion in April 2012. Over 40 different models with 439 WSI were reviewed including GEM, xenografted, rat and canine models. The major models included Pten/Akt, Rb/p53, Myc and Erg pathways. The panel identified, defined and classified newer types of lesions including: epithelial hyperplasia, neuroendocrine tumors, EMT (sarcomatoid carcinomas), intra-cystic adenocarcinomas, prostatic metastases and high grade PIN. The current work will provide a consensus report updating the research community on current pathological

analysis of GEM and providing a detailed description of important new GEM models. The use of digital imaging proved effective and a valuable resource. The entire WSI collection will be available for public viewing fall 2012.

BONE METABOLISM BIOMARKERS IN CASTRATION RESISTANT PROSTATE CANCER (CRPC) PATIENTS WITH SKELETAL METASTASES: RESULTS FROM SWOG 0421

Primo N. Lara, Jr., Benjamin Ely, David I. Quinn, Cathy Tangen, Erik Gertz, Maha Hussain, Nicholas J. Vogelzang, Ian Thompson, and Marta Van Loan

Background. Prior studies suggest that elevated markers of bone turnover are prognostic for poor survival in CRPC. The predictive role of these markers relative to bone-targeted therapy is unknown. We prospectively evaluated the prognostic and predictive value of bone metabolism markers in sera from CRPC patients treated on a placebo-controlled phase III trial of docetaxel with or without the bone targeted endothelin-A receptor antagonist atrasentan (SWOG S0421).

Methods. Markers for bone resorption (N-telopeptide [NTx] and Pyridinoline [PYD]) and formation (C-terminal collagen propeptide [CICP] and bone alkaline phosphatase [BAP]) were assayed in pre-treatment and serial sera. Cox regression models for survival based on marker levels adjusted for clinical variables were developed. A Cox model was fitted with main effects and marker-treatment interaction, adjusted for clinical variables, to assess predictive value of atrasentan on survival. Analysis was adjusted for multiple comparisons.

Results. Sera from 778 patients were analyzed. Elevated baseline levels of each of the markers was associated with worse survival ($p<0.001$), as shown in the table below.

Biomarker	**Hazard Ratio** (95% CI)	**Median Survival, months** (<= median BMB value vs. > median)	**p-value***
BAP (u/l)	1.26 (1.19, 1.35)	22.8 vs. 15.4	<0.001
CICP (ng/ml)	1.38 (1.27, 1.5)	24.5 vs. 14.6	<0.001
NTx (nM)	1.43 (1.31, 1.57)	22.4 vs. 15.5	<0.001
PYD (nmol/l)	1.49 (1.32, 1.7)	20.3 vs. 15.3	<0.001
*Significance was set at $\leq$ 0.006 (Bonferroni adjustment to control overall 2-sided error rate across 4 tests at 0.05). **For analysis of BMB x Treatment interaction, adjusted for clinical variables, significance was set at <0.01 to control overall 2-sided error rate at 0.05 (for the two specified follow-up analyses using marker cutpoints at the median and, alternatively, at the upper 25%-ile).			

Increasing marker levels by week nine of therapy were also associated with subsequent poor survival ($p<0.001$). Patients with the highest marker levels (upper 25th percentile) not only had a poor prognosis (hazard ratio [HR] = 4.3, $p<0.001$) but had a survival benefit from atrasentan (HR=0.33, median survival = 13 vs. 5 months; interaction $p=0.005$).

Conclusions. Serum bone metabolism markers have significant independent prognostic value in CRPC. Importantly, a group of CRPC patients with highly elevated markers of bone turnover were identified that preferentially benefits from bone-targeted therapy.
(5R01-CA120469; ClinicalTrials.gov: NCT00134056)

THE ROLE OF MXD3 IN HUMAN PRECURSOR B CELL ACUTE LYMPHOBLASTIC LEUKEMIA

Gustavo Barisone[1], Noriko Satake[2], Carly Lewis[3], Kit Lam[4], Jan Nolta[5], Elva Diaz[1]

[1]Department of Pharmacology, UC Davis, [2]Department of Pediatrics, UC Davis, [3]Carver College of Medicine, University of Iowa, [4]Department of Biochemistry and Molecular Medicine, UC Davis, [5] Stem Cell Program, UC Davis

MXD3 is a transcription factor previously shown in our lab to be a novel member of the Hh pathway (Yun, Rust, Ishimaru, Diaz, Mol Bio Cell, 2007). We have previously shown that MXD3 is expressed in medulloblastoma and that its knockdown reduces proliferation of human medulloblastoma cell lines (Barisone et al., PLoS, 2012). In the current study, we investigated a possible role for MXD3 in precursor B (preB) acute lymphoblastic leukemia (ALL) cell proliferation. Using qRT-PCR we observed 13 to 35 times higher levels of MXD3 mRNA expression in 8 primary preB ALL samples, as well as in the preB ALL cell lines Reh and JM1, than in mobilized peripheral blood mononuclear cells from healthy donors, mouse bone marrow and spleen. Immunoblot analysis with anti-MXD3 monoclonal antibodies confirmed that the protein was present in the ALL samples but not in normal cells. We investigated the role of MXD3 in cell proliferation and survival by silencing MXD3 in the Reh cell line. We used lentiviral delivery to knockdown MXD3 using an RNA interference approach. Upon transduction by the viral vector, MXD3 knockdown was confirmed at both the RNA and protein level. Within 48 hours, MXD3 protein levels were reduced >90% in cells infected with the shMXD3 virus but not with the control virus. MXD3 knockdown resulted in decreased proliferation in Reh cells, supporting our hypothesis that it may be involved in the maintenance of ALL. To understand the role of MXD3 in leukemia cells, we analyzed cell cycle progression and apoptosis levels after knockdown using flow cytometry. We observed no significant differences in the G0/G1, S or G2/M populations between experimental and control samples. However, samples where MXD3 had been knocked down showed higher levels of apoptosis when compared to controls.
Our results suggest that MXD3 is important for preB ALL cell proliferation, possibly by acting as an anti-apoptotic factor. Therefore, MXD3 is a potential candidate for targeted therapies against preB ALL.

OVERCOMING RESISTANCE TO TYROSINE KINASE AND ANDROGEN RECEPTOR TARGETED THERAPIES IN CASTRATION-RESISTANT PROSTATE CANCER.

Yang JC, Nguyen H, Wu Z, Chang P-C, Chu C-Y, Wang L-Y, Chen N-T, Ma A-H, Desai SJ, Lo SH, Lam KS, Kung H-J, Evans CP.

Departments of Urology, Biological Chemistry and Molecular Medicine, and Internal Medicine, University of California at Davis School of Medicine and UC Davis Comprehensive Cancer Center.

Macroautophagy (hereafter referred to autophagy) is an evolutionarily conserved process designed to degrade long-lived proteins and organelles to maintain homeostasis. Under cellular stress conditions, autophagy is rapidly upregulated, providing an alternative source of energy to enable continuous cell survival. Excessive or unquenched autophagy however, can lead to type II programmed cell death, which is morphologically distinct from apoptosis and usually caspase-independent. Defective autophagy may contribute to tumorigenesis, while functional autophagy in response to chemotherapy may lead to chemoresistance of different carcinoma cells. This talk will demonstrate a role for autophagy in two contexts: mediating resistance to Src and androgen receptor inhibitors.

We've demonstrated that Src kinase is activated in prostate cancer, conferring castration-resistance and stimulating metastasis and intracrine androgen biosynthesis. Others have shown that activated Src in CRPC patient tumors correlates with resistance to enzalutamide (MDV3100). We have reported a role for Src activity in the suppression of autophagy and the novel finding that Src inhibitors such as PP2 and AZD0530 (saracatinib) effectively induce autophagy in CaP cells, as does siRNA targeted inhibition of Src expression. The Src inhibitor-induced autophagy is accompanied by the inhibition of the PI3K (type I)/Akt/mTOR signaling pathway. To test whether autophagy blockade could lead to enhanced cell death, pharmacological inhibitors (3-methyladenine and chloroquine) and a genetic inhibitor (siRNA targeting Atg7) were used in combination with Src inhibitors. The results show that autophagy inhibition effectively enhanced cell killing induced by Src inhibitors. Importantly, a combination of saracatinib with chloroquine in mice significantly reduced prostate cancer (PC3) xenograft growth compared with controls. We then sought to optimize Src inhibitor therapy in patients, as a Phase II trial did not demonstrate significant tumor

apoptosis and PSA response. To do this, bone marrow biopsies of patients with CRPC will undergo an AR/Src sensitivity gene signature analysis. Patients with the Src sensitivity gene signature will be treated with a Src and autophagy inhibitor.

With regard to antiandrogen therapy, we investigated whether autophagy is involved with development of resistance to bicalutamide or enzalutamide. We found that the autophagic cascade is triggered by AR blockade and resulted in a subpopulation of enzalutamide resistance C4-2B cells. The autophagy was mediated by AMPK activation and suppression of mTOR through Raptor phosphorylation. Si-RNA targeting of AMPK inhibited autophagy and promoted cell-death. These observations are now undergoing in vivo testing and development of a dual AR/autophagy inhibitor clinical trial.

ABSTRACTS OF ORAL PRESENTATIONS: FRIDAY

SESSION III. DEVELOPMENTS IN NUTRITION AND CANCER

LET'S DEPERSONALIZE MEDICINE

Richard Levensen, MD

Tremendous efforts to appreciate the complexity of cancer and the specific threats and vulnerabilities of individual malignant tumors are underway, involving large-scale and high-resolution DNA, RNA and protein characterization. Results of these investigations may shape the kinds of tissue-based analytical tools that anatomic pathology will be called upon to develop and deploy. Increasingly, these potentially multiplexed panels of molecular assays will prove to be complex, expensive, proprietary, and hard to independently validate. But what if, in addition, these tests turn out to be focused on the trees and in fact are missing the forest: emerging insights into the interplay between cancer and host, and new appreciation of the impact of stroma and of host genomic factors in individual tumor evolution, may allow us to better understand the underlying processes at work. Such insights can lead to simpler and lower cost assays with improved impact on prognostic accuracy, treatment selection and outcome.

MILK, THE MICROBIOTA AND CANCER: WHAT CAN EVOLUTION TEACH US?

J. Bruce German[1,2]

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The appropriately educated, assembled and activated immune system is the secret of our survival through our chemically and microbially hostile environment. Research is discovering that diverse immune processes are in turn reacting to environmental cues as the means to coordinate surveillance, response and repair. Maddeningly, but perhaps not surprisingly, this dynamic communication system is the key to survival but it can also be the engine of damage when dysfunctional. For example insufficient activation of the immune system is associated with failure to respond to infectious pathogens and inappropriate stimulation leads to actions, collectively termed chronic inflammation, that produce insidiously damaging effects to molecules, tissues and processes throughout the body. Chronic inflammation appears to be responsible for accelerating all of the major degenerative diseases afflicting humans. If balance is the key to appropriate immune function, how do we determine what is effective stimulation and destructive dysregulation? We have taken an explicitly evolutionary approach to examining nutrition in this context. Mammalian lactation evolved over 200 million years with the relentless Darwinian pressure to be not only nourishing but supportive of the development and functions of the naïve, neonatal immune system. Milk supports infant immune development and is yet highly anti-inflammatory. Milk selects and guides a very specific microbiota in the infant consisting of a remarkable predominance of the single bacterium Bifidobacteria longum biovar infantis. Milk components bind chemical and microbial toxins, deliver nutrients, enhance barrier functions and regulate cell growth. Immunity and cancer are intimately related and in fact, most cancers are not possible without immune dysfunction. All of cancer's various steps: from cellular damage to metastatic tissue invasion are accelerated by the types of collateral damage associated with chronic inflammation. Also, immune surveillance is necessary for removal of disregulated cells including tumorigenic cells. The principles from lactation biology provide the means to approach cancer from multiple new directions.

FOUR HORSEMEN: AGING, OBESITY, INFLAMMATION, AND MICROPHAGES

William Murphy, PhD. Acting Chair, Department of Dermatology, UC Davis School of Medicine.

Cancer commonly occurs in the elderly, a population in which immune therapy is also being increasingly applied. We assessed the impact of age on responses to systemic immune stimulation. In contrast to young mice, immunostimulatory regimens given to aged-mice resulted in rapid and lethal toxicities affecting numerous organs correlating with heightened pro-inflammatory cytokines (TNFα/IL6) and body fat. Caloric-restricted aged-mice had markedly reduced cytokine levels and protection from pathology. Increased tissue TNFR expression was also observed with aging. *In vivo* depletion of macrophages in aged-mice resulted in lesser cytokine levels and complete protection from pathology. Macrophages from elderly volunteers displayed higher TNF production. Finally, *in vivo* TNF blockade in tumor-bearing aged-mice given immunotherapy resulted in increased survival due to protection from toxicity and induction of anti-tumor effects demonstrating an intricate relationship between TNFα, macrophages and body fat as factors in the age-associated pathologic responses to systemic immune stimulation.

VITAMIN A AND LIVER HEALTH

Yuqi He and Yu-Jui Yvonne Wan

Department of Pathology and Laboratory Medicine, University of California, Davis

The eye and skin are obvious retinoid target organs for vitamin A. However, vitamin A is stored and processed in the liver. The binding proteins are produced by the liver and the receptors for retinoic acid are expressed in the liver. Surprisingly, the action of vitamin A in the liver is unknown. To study retinoic acid (RA)-mediated effect in the liver, we first produced liver specific retinoid x receptor α (RXRα)-deficient mice and showed that serum triglyceride and cholesterol levels were elevated due hepatic RXRα deficiency. In addition, lack of hepatic RXRα increased sensitivity to alcohol- and non-alcohol-induced steatosis and steatohepatitis. Furthermore, RXRα-mediated signaling is altered in HCV-infected human livers. These findings indicated the effect of liver RXRα in regulating lipid homeostasis and inflammatory pathways.

By chromatin immunoprecipitation using anti-RXRα and -RARα antibodies follow by sequencing (ChIP-seq), we identified genome-wide binding of these two nuclear receptors in mouse liver. We have also identified differential hepatic gene expression between wild type and hepatocyte RXRα-deficient mice by microarray. By combining these two datasets, the relationship between genomic binding and hepatic gene expression was established. Based on the binding data generated in our study and published data, we showed the global relationship between hepatic RXRα and other nuclear receptors and defined their potential biological actions. The data showed a close relationship between RXRα and PXR, LXR, FXR, and PPARα in regulating lipid homeostasis. To further explore the action of RXRα and RARα as well as their ligand RA in regulating lipid homeostasis in the liver, the expression pattern of 576 genes that regulate lipid homeostasis in the KEGG pathway was profiled in wild type and liver RXRα knockout mice treated with and without RA. The data showed that RA treatment and RXRα-deficiency had an opposite effect in regulating lipid homeostasis. A subset of genes (114) which could clearly differentiate the effect of ligand treatment and receptor deficiency were selected for further functional analysis. The data indicated that RA treatment produces unsaturated fatty acids, induces triglyceride breakdown, bile acid secretion, and lipolysis, as well as retinoids elimination. In contrast, RXRα deficiency causes the synthesis of saturated fatty acids, triglyceride, cholesterol, bile acids and retinoids. Binding data indicate extensive cross-talk among RARα, PXR, LXR, FXR, and PPARα in regulating those RA/RXRα-dependent genes. Moreover, biochemical data proved that RA could lower serum cholesterol, triglyceride, and bile acid level in mice. Taken together, RA, the active metabolite for vitamin A, mediated via nuclear receptors has extensive role in regulating liver metabolism in general. The action of RA in relationship with bile acid homeostasis and intestinal microbiota will be discussed in the presentation.

DIET, IMMUNITY AND INFLAMMATION

Charles Stephensen, Ph.D.

Diet affects the immune system in many ways. It provides essential nutrients needed for immune cell survival and proliferation (e.g., amino acids, energy), it provides key components for signaling within and between immune cells (e.g., minerals, vitamins A and D), and it directly affects the composition of the gut microbiome (e.g., iron, fiber) which itself interacts with the mucosal and systemic immune response. For these reasons diet is an important consideration both in the prevention and treatment of chronic inflammatory conditions, including some cancers. In particular, some dietary components that may be low in the US diet may promote a regulatory rather than inflammatory response and thus correcting deficiencies may diminish rather than enhance damaging inflammation. In particular, increasing intake of nutrients such as vitamin D and omega-3 fatty acids may have direct anti-inflammatory benefits, while increasing intake of probiotics may indirectly affect inflammation by affecting the gut microbiome.

BRINGING SYNBIOTIC INTERVENTIONS IN CANCER TO PRACTICE: FROM BENCH TO BEDSIDE

Jennifer T. Smilowitz[1,2]

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Altered gut microbiota is linked to inappropriately activating the innate and adaptive immune responses, impairing gut integrity and increasing chronic inflammation which contribute in turn to carcinogenesis and disease progression. Probiotics, consumed as living bacteria, have been shown to exert health effects; however, these effects have been modest which is possibly due to the choice of probiotic bacteria, historically governed by convenience and availability rather than function. Similarly, prebiotics, consumed as indigestible fermentable carbohydrates are typically simple polymers that can be fermented by virtually all bacteria including overt pathogens but not necessarily only the beneficial strains. Leveraging from our research in human lactation, we now know that human milk provides a model for how to develop a healthy microbiome and the intestinal environment it shapes. Human milk delivers a complexity of milk bioactive components with structure-function properties that underlie its benefits to the vulnerable, immune-naïve neonate. In particular, Bifidobacteria longum subsp. infantis, a strain of bacteria found in the GI tract of exclusively breast-fed infants, aids in the degradation and structural modification of enteral antigens, regulates the secretion of inflammatory mediators, increases the integrity of the GI tract and directs the development of the immune system. Refashioning the intestinal microbiome to reduce inflammation, improve GI integrity, and immune responsiveness is an attractive dietary strategy to thwart the etiology and progression of a number of different types of cancers and improve the quality of life during cancer treatment. Translating the benefits of human lactation to adults with inappropriately responsive immune systems is possible with novel methodological strategies using bovine dairy streams. These synergistic dietary solutions have the potential to appropriately activate the immune system, reduce inflammation and improve gastrointestinal function when delivered as adjuvants before, during and after the administration of cancer therapies.

Key Words
Inflammation, Immunity, Probiotics, Oligosaccharides, Colostrum

POSTER PRESENTATIONS

Courtyard by Marriott Hotel

4422 Y Street, Sacramento

Poster Index:

Poster abstracts:

Thursday poster session: 11:20 am – 12:50 pm
Posters can be put up from 7:30 am
Posters must be taken down by 1:30 pm

Friday poster session: 9:00 am – 11:00 am
Posters can be put up from 7:30 am
Posters must be taken down by noon

THURSDAY POSTER PRESENTATIONS (INDEX)

1. **Characterizing the Effect TIN2 Mutations have on Telomerase Activity: Amplification and Sequence Analysis of Endogenous and Engineered TIN2**
   Sara Balla, Amanda Frank, Lifeng Xu
2. **Gene deletion underlies loss of p16 expression in osteosarcoma tumors with poor response to neoadjuvant chemotherapy**
   Dariusz Borys, Robert Canter, Jeffrey Gregg, Ben Hoch, Ryan Davis, Andrew Horvai.
3. **Expression of p16 in osteosarcoma as predictive neo-adjuvant therapy factor**
   Dariusz Borys, Robert Canter, Robert Tamurian, Benjamin Hoch, Brian Murphy, John Bishop and Andrew Horvai.
4. **Crystallization of *E. Coli* MutY bound to DNA to investigate structural and catalytic function of serine120**
   Castillo SIP, Woods RD, David SS
5. **The effect of MM-121 on ErbB3 and the transitional cell carcinoma cell line TCCSUP**
   Duanna Challenger, Benjamin A. Mooso & Paramita M. Ghosh
6. **Increased eIF4E phosphorylation prevents response of prostate cancer cells to mTOR inhibitors**
   LS D'Abronzo, RE Beggs, S Bose, PM Ghosh
7. **Genomic profiling of non-small cell lung cancer patient-derived xenograft models for personalized cancer therapy**
   Sonal J. Desai, Neal Goodwin, Regina Gandour-Edwards, David R. Gandara and Tianhong Li
8. **Regulation of oncogenic EZH2 by ANCCA/ATAD2 and androgen in prostate cancer**
   Zhijian Duan, June X. Zou, Ping Yang, Yuzhuo Wang, Alexander D. Borowsky, Allen C. Gao, and Hong-Wu Chen
9. **Treatment for refractory brain metastases in patients with EGFR mutation-positive lung cancer**
   Hanh La, Sonal J. Desai, Krysteena Tolentino, Joyce Lee, Wenwu Xiao, Kit S. Lam, and Tianhong Li
10. **Stabilization and sequestration of the ubiquitin ligase Nrdp1 by the reticulon Rtn4a**
    Jason Hatakeyama, Hanine Rafidi, Colleen Sweeney, and Kermit L. Carraway, III
11. **Repression of Cyclin G2 blunts the cell cycle arrest response of MCF-7 cells to antagonists of estrogen receptor signaling and the AMPK activator metformin.**
    Zimmermann, M., Arachchige Don, A.S., Donaldson, M.S., Horne M.C.
12. **ErbB3 overexpression induces resistance to dual EGFR/ErbB2 inhibitors in prostate cancer**
    Maitreyee K. Jathal, Benjamin A. Mooso, Leandro S D'Abronzo, Salma Siddiqui, Liqun Chen, Margarita Mikhailova, Elyse van Spyk and Paramita M. Ghosh
13. **Comparison of the effects of four ErbB inhibitors in two bladder cancer cell lines expressing high and low levels of these receptors**
    Benjamin A. Mooso, Paramita M. Ghosh
14. **The androgen receptor modulates p14arf expression in human prostate tumor and tumor derived cell lines**
    Salma Siddiqui, Mohana Roy, Frank Melgoza, Alexander Borowsky, Stephen J. Libertini, LiHong Qi, Karim Chamie, Stephanie Soares, Benjamin Mooso, Paramita M. Ghosh, and Maria Mudryj
15. **Androgen receptor-mediated regulation of non-coding RNAs in two castrate resistant cells lines- an additional layer of complexity**
    Stephen J. Libertini and Maria Mudryj
16. **Synthesis of martinelline and martinellic acid: potential inhibitors of colon cancer cell proliferation**
    Manuel Muñoz, Nohemy Sorto, Jared T. Shaw

17. **Induction of rapid telomere shortening by elevated levels of TRF2**
    Bernadette Nera, Annie Chiu, Lifeng Xu

18. **Higher baseline TNF receptor and TNF-α expression in macrophages of aged mice**
    Axana Rodriguez-Torres, Gail D. Sckisel, Annie Mirsoian, and William J. Murphy

19. **N-glycan profiles as potential markers for the early detection of lung cancer**
    L.Renee Ruhaak, Suzanne Miyamoto, Carol Stroble, Matt Barnett, Karen Kelly, Ziding Feng, Samir Hanash, David Gandara and Carlito B. Lebrilla

20. **Tumor inoculation with *E. Aerogenes* results in prolonged survival in a rat glioblastoma model: initial proof of concept for intracranial probiotic therapy.**
    Whitney K. Cheung, J. Paul Muizelaar, Rudolph J. Schrot.

21. **The effects of MM-121 on androgen dependent and independent prostate cancer cells**
    Thomas Steele, Maitreyee K. Jathal, Paramita M. Ghosh

22. **Utilization of transcriptome sequencing for the identification of blood-based biomarker signatures of hepatocellular carcinoma in HBV-infected Asian Americans.**
    Clifford G. Tepper, Richelle L. Enriquez, Jeffrey P. Gregg, Christopher L. Bowlus. Ryan R. Davis, Stephenie Y. Liu, Julie Dang, Susan L. Stewart, and Moon S. Chen, Jr.

23. **A new family of pluripotency-related oncogenes**
    Po-Yuan Tung, Natasha Varlakhanova, Paul Knoepfler

24. **Transduction and transplantation of mammary epithelial cells to explore Nrdp1 function**
    Jessica Wald, Jason Hatakeyama, David Boucher, Colleen Sweeney, Russell Hovey, & Kermit Carraway, III

25. **Histone methylase NSD2 drives endocrine therapy resistance by stimulation of multiple kinase signaling pathways**
    Junjian Wang1, Zhijian Duan1, June X. Zou1, Alexander D. Borowsky2, and Hong-Wu Chen1

26. **Inhibiting Hedgehog signaling activity suppressed the tumorigenicity, proliferation and metastasis of hepatoma subpopulations**
    Samantha Nguyen, Akshita Verma, Akshay Kashyap, Sepehr Hashemi, Tsung-Chieh Shih, Jian Wu.

27. **Synergistic combination of microneedles and biochemical approaches for intra-tissue delivery of oligonucleotides in living cells in 3D tissue phantoms**
    Ting Ye, Zhen Luo, Yunzhe Ma, Harvinder S. Gill, Nitin Nitin

28. **Roles of non-proteinaceous lipid mediators in angiogenesis**
    Zhang Guodong et al.

FRIDAY POSTER PRESENTATIONS (INDEX)

17. **Functional p53 determines docetaxel sensitivity in prostate cancer cells**
    Chengfei Liu, Yezi Zhu, Wei Lou, Nagalakshmi Nadiminty, Xinbin Chen, Xubao Shi, Ralph W. deVere White, and Allen C. Gao¶
18. **Androgen Responsive miR-148a Alters Cell Cycle Regulation by Targeting p27 in Prostate Cancer Cell Lines**
    Alan P. Lombard, Stephen J. Libertini, Alyssa C. Thunen, Keeley B. Fornaci, Maria Mudryj
19. **Stability of miRNA in human urine supports their biomarker potential in urologic cancers**
    Christine Mall, David M. Rocke, Blythe Durbin-Johnson, and Robert H. Weiss
20. **Lrig3 promotes Wnt signaling in breast cancer cells through stabilization of the Wnt co-receptor, Lrp6**
    Frank Mercado, Michael Astudillo and Colleen Sweeney
21. **Social and Cultural Influences on Tobacco-Related Health Disparities among South Asians in the United States**
    Arnab Mukherjea
22. **Moving Towards a True Depiction of Tobacco Use among South Asians: Analyses from the California Asian Indian Tobacco Use Survey**
    Arnab Mukherjea, Mary V. Modayil, Elisa K. Tong
23. **Computational modeling and analysis of negative feedback and coupled signaling in the Smad-dependent TGF-β signal transduction pathway**
    Daniel Nicklas and Leonor Saiz
24. **Identification of Nrdp1 as a novel androgen receptor transcription target differentially regulated in androgen-dependent and independent prostate cancer**
    Rosalinda M. Savoy, Liqun Chen, Salma Siddiqui, Frank Melgoza, Mohana Roy, Ryan E. Beggs, Maitreyee K. Jathal, Swagata Bose, Yu Wang, Roble G. Bedolla, Dean A. Troyer, Margarita Mikhailova, William H. Fry, Kermit L. Carraway, III, Paramita M. Ghosh.
25. **Immunostimulatory Cancer Immunotherapy Regimens Induce Subsequent Potent Immunosuppressive Responses**
    Gail Sckisel, Myriam Bouchlaka, Annie Mirsoian, Hui-Hua Hsiao, Arta Monjazeb, William J. Murphy
26. **In vivo evaluation of the growth inhibitory function of Lrig1 in the mammary gland**
    Catalina Simion, Jane Q. Chen, Charles Wilkerson, Hanine Rafidi, Alexander D. Borowsky and Colleen Sweeney
27. **Role of H2-Relaxin in inducing radiation resistance through p53 pathway**
    Alaric B. Smith, Ruth L. Vinall, Sheetal Singh, Jean Cheung, Clifford G. Tepper, Maria Mudryj, Ralph W. deVere White, Paramita M. Ghosh
28. **Prostate Epithelium-Specific R270H Mutation in the p53 Gene Induces Prostatic Intraepithelial Neoplasia in Mice**
    R. L. Vinall, J. Q. Chen, N. E. Hubbard, S. S. Sulaimon, R. W. DeVere White, and A. D. Borowsky
29. **COLLECTIVE PROSTATE CANCER CELL INVASION DEPENDS ON N-CADHERIN**
    Yuanyuan Cui and Soichiro Yamada

THURSDAY POSTER PRESENTATIONS (ABSTRACTS)

<<1>>

CHARACTERIZING THE EFFECT TIN2 MUTATIONS HAVE ON TELOMERASE ACTIVITY: AMPLIFICATION AND SEQUENCE ANALYSIS OF ENDOGENOUS AND ENGINEERED TIN2

Sara Balla, Amanda Frank, Lifeng Xu

Department of Microbiology, University of California at Davis, Davis, CA 95616

Patients with Dyskeratosis Congenita (DC) are at high risk for developing bone marrow failure and multiple types of cancer. All DC patients have extremely short telomeres, regions of repetitive sequence at eukaryotic chromosome ends. In most human somatic cells, telomeres shorten with each round of cell division. However, in cancer, stem, and germ line cells, the telomeres are elongated by the multi-subunit reverse transcriptase telomerase. Telomerase access to the telomeres is regulated by the telomere-binding shelterin protein complex. Mutations within the subunits of telomerase have been found in some patients with DC. Recently, however, heterozygous mutations within the shelterin component TIN2 have been discovered in DC patients that do not have telomerase mutations. It is unclear why the mutations within TIN2 lead to short telomeres in DC patients. We hypothesize that the TIN2 mutations affect telomerase function resulting in short telomeres. Previously, it was determined that the TIN2 mutations do not function in a dominant negative manner. To test if the TIN2 mutations are haploinsufficient, human cell lines with heterozygous TIN2 mutations were created by gene editing. PCR (polymerase chain reaction) was performed to amplify both the endogenous TIN2 allele and the edited mutant or wildtype TIN2 allele. The TIN2 sequence was then analyzed to verify that there were no additional mutations. Thus far, the sequences for six wildtype and two mutant clones have been confirmed with no other mutations than the one we engineered. After verifying the TIN2 sequence, we plan to compare telomere length and telomerase activity of the mutant and wildtype clones to gain insight into the mechanism by which TIN2 mutations lead to short telomeres.

The following abstract submission is for a poster presentation at the 17th Annual Cancer Research Symposium. I am a part of the CURE (Continuing Umbrella of Research Experience) program.

<<2>>

GENE DELETION UNDERLIES LOSS OF P16 EXPRESSION IN OSTEOSARCOMA TUMORS WITH POOR RESPONSE TO NEOADJUVANT CHEMOTHERAPY

Dariusz Borys, Robert Canter, Jeffrey Gregg, Ben Hoch, Ryan Davis, Andrew Horvai.

University of California Davis, Sacramento; UC Davis, Sacramento; University of Washington, Seattle; UCSF, San Francisco

Background: Although pathologic response to neoadjuvant chemotherapy predicts survival among patients with osteosarcoma (OS), there are currently no established molecular markers to predict response to chemotherapy. We have previously shown that immunohistochemical (IHC) expression of p16 (the product of the CDKNA2 gene) in OS is significantly correlated with pathologic response to neoadjuvant chemotherapy. The objective of the current study was to assess for copy number alternations at the CDKNA2 gene on chromosome 9 in p16 IHC-expressing and IHC-non-expressing OS specimens.
Design: Genomic DNA was obtained from paraffin-embedded pretreatment biopsy specimens of OS patients prior to receiving neoadjuvant chemotherapy. We selected three cases of p16 IHC-expressing tumors with pathologic response to chemotherapy (≥ 90% tumor necrosis) and three cases of P16 IHC-non-expressing tumors without pathologic response (< 90% tumor necrosis). Human genomic array was performed on 44K arrays using 80 Gb per human genome at 30X coverage. The CDKN2A locus (p16) on chromosome 9 was probed with A_14_P129522 chr9, A_14_P130650 chr9 and A_14_P112983 chr9.
Results: Four of the 6 cases yielded complete copy number information (2 p16 IHC-expressing, 2 p16 IHC-non-expressing). Among p16 expressing tumors, both demonstrated a wildtype CDKNA2 locus, while one of two p16 non-expressing tumors had a deletion on the short arm of chromosome 9 including the CDKNA2 locus.
Conclusions: Deletion of the entire CDKNA2 locus explains loss of p16 expression in some OS patients with poor response to neoadjuvant chemotherapy. However diverse mechanisms may be responsible for

loss of p16 expression across the population of OS patients. Additional molecular studies are needed to validate these findings.

<<3>>

EXPRESSION OF P16 IN OSTEOSARCOMA AS PREDICTIVE NEO-ADJUVANT THERAPY FACTOR

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Background: Osteosarcoma (OS) is a common malignant primary tumor of bone affecting adolescent and young adults. Osteosarcomas are high grade sarcomas with aggressive behavior. There are few if any molecular markers to predict behavior and prognosis of osteosarcoma. The objective of this study is to investigate expression of p16 in correlation with neo-adjuvant chemotherapy response in osteosarcoma.

Design: A tissue micro array was created using paraffin embedded samples from 40 pretreatment osteosarcoma cases from two institutuions UC Davis and UCSF. Immunohistochemistry was performed with commercially available p16 monoclonal mouse antibody (mtm laboratories AG, Germany). Expression for p16 was defined as nuclear staining in at least 10% of cells. Percent tumor necrosis was measured in post-chemotherapy resection specimens with good response set at >90% necrosis.

Result: Patients age ranged from 9 to 75 years (mean 20). Most common locations were tibia and femur. 21 patients were female and 19 male. The clinical and p16 results are summarized in table 1. P16 expression correlated positively with median percent necrosis and fraction of cases with good chemotherapy response (p=0.004 and 0.003, respectively).

Conclusion: Immunohistochemical expression of p16 significantly correlates with good chemotherapy response in osteosarcomas. p16 immunohistochemistry may be useful adjunctive marker of prognosis in osteosarcoma.

TABLE 1. Comparison of Clinicopathologic Characteristics among p16 Over- and Under-Expressing Tumors

Characteristic	p16 Positive (N=25)	p16 Negative (N=15)	P Value
Median Age, IQR	15,12-21	17, 13-26	0.51
Sex: Male/Female	15 (60%)/10 (40%)	4 (27%)/11 (73%)	0.06
Median Percent Necrosis, IQR*	95/ 90-99	40/ 10-90	0.004
Pathologic "good" response (>90%)* : Yes/ No	18 (78%)/ 5 (22%)	4 (27%)/ 11 (73%)	0.003

*Indicates statistically significant difference

<<4>>

CRYSTALLIZATION OF E. COLI MUTY BOUND TO DNA TO INVESTIGATE STRUCTURAL AND CATALYTIC FUNCTION OF SERINE120

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The DNA glycosylase MutY is a critical component of the 8-oxo-7,8-dihydro-guanine (8-oxoG) base excision repair (BER) pathway. MutY excises adenine from the DNA duplex when misincorporated opposite 8-oxoG during replication. Downstream BER enzymes complete the repair after correct pairing with cytosine. Inherited mutations in the human gene for the MutY homolog, MUTYH, have been linked to colorectal cancer, termed MUTYH-Associated Polyposis (MAP). Recently, crystal structures of *B. stearothermophilus* MutY (BsMutY) have suggested that a widely conserved Tyr126 residue is crucial to catalytic function (unpublished). The Tyrosine's location in the MutY active site implies that it is a participant in the catalytic mechanism of adenine excision. However, the *E. coli* MutY homolog contains a Serine at the equivalent position. It is not certain that this Ser120 residue plays the identical role in enzymatic function due to the differing position of the hydroxyl group compared to that of Tyr126 in BsMutY. Furthermore, kinetic studies of the BsMutY analogue containing the conserved tyrosine have

shown that a Tyr→Ser point mutation causes catalytic deficiency (unpublished). To ascertain the catalytic function of Ser120 in E. coli MutY, wild-type and truncated forms bound to DNA containing a transition state analogue will be crystallized via hanging drop method. High quality crystals will be analyzed via x-ray diffraction.

<<5>>

THE EFFECT OF MM-121 ON ERBB3 AND THE TRANSITIONAL CELL CARCINOMA CELL LINE TCCSUP

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There are estimated to be about 73,000 new cases of bladder cancer each year and about 14,000 deaths. Transitional cell carcinoma accounts for 90% of those cases. Treatment may involve surgical removal of the bladder, alone or together with radio or chemotherapy, which hinders the quality of life for the patients. Post-treatment recurrence is observed in a fraction of patients with muscle-invasive (MI) bladder cancer. The purpose of our research is to prevent recurrence in patients with MI disease. Recurrence occurs due to resistance of the bladder cancer cells to chemotherapy. Recurrent bladder cancer cells are found to express higher levels of the epidermal growth factor receptor (EGFR) and related receptors ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4), hence a number of ErbB inhibitors have been tried in patients with bladder cancer but the results are not encouraging. However, previous studies in other tumor types demonstrated that resistance to EGFR and ErbB2 inhibitors arise due to a concomitant increase in ErbB3 levels, hence we investigated whether MM-121, a monoclonal antibody inhibiting ErbB3, sensitized bladder cancer cells to chemotherapy. Investigation was conducted in TCCSUP and J-82 cells with high ErbB expression, T-24 cells with intermediate ErbB expression and HTB-2FT4 cells with low ErbB levels. Comparison of growth curves of the various cell lines indicated that the IC50 value for J-82 was about 0.86 μM, whereas that for TCCSUP and HTB-2FT4 cells were about 10 μM, while T-24 cells were resistant to the drug. However, in all 4 cases, MM-121 completely or partially sensitized the cells to 200 nM Cisplatin. In addition, MM-121 in combination with dual EGFR/ErbB2 inhibitors lapatinib and dacomitinib, severely inhibited cell survival. We have shown that when ErbB3 is deactivated, its downstream pathway can still be activated through heterodimerization with EGFR. Thus, it would be more effective to block multiple receptors of the EGFR pathway to prevent heterodimerization that leads to cell proliferation and DNA synthesis.

Acknowledgements: We thank Pfizer, Inc. for the gift of dacomitinib. This work was supported by a Development Grant from the UC Davis Cancer Center.

<<6>>

INCREASED EIF4E PHOSPHORYLATION PREVENTS RESPONSE OF PROSTATE CANCER CELLS TO MTOR INHIBITORS

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We previously showed that the mTOR pathway is activated in castration resistant prostate cancer (CRPC) while its inhibition upregulated androgen receptor (AR) signaling, resulting in resistance to mTOR-based therapy. In this study we used RAD001, an mTORC1 inhibitor, BEZ235, a dual mTOR/PI3K inhibitor and Torin1 that inhibits both mTORC1 and mTORC2. Comparison of the effects of RAD001, alone, or in combination with the AR inhibitor bicalutamide in various cell lines showed that although C4-2 cells responded well to the combination, other cell lines, such as LNCaP-AI, PC-346C and CWR-R1, did not. The resistance of LNCaP-AI and CWR-R1 was partially overcome by treatment with BEZ-235 and Torin1, alone or in combination with bicalutamide; however, PC-346C cells were resistant to both drugs. Hence, the molecular effects of the drugs in downstream targets p70S6 kinase, 4E-BP1, eIF4G, Akt and eIF4E were compared in C4-2 and PC-346C. Western blots revealed that p70S6 kinase and 4E-BP1 phosphorylation were effectively downregulated by BEZ235, but not Torin1, compared to RAD001. Inactivation of the translation initiation complex requires inhibition of eIF4E. In C4-2 cells, eIF4E phosphorylation was increased by RAD001 but suppressed by the other two drugs. However, in PC-346C cells, despite inhibition of 4E-BP1 phosphorylation, eIF4E phosphorylation was increased and this may be

the reason why PC-346C cells are resistant to mTOR inhibitors. Our data indicate eIF4E phosphorylation as a possible cause of resistance to mTOR inhibitors, hence pre-screening patients to identify those with high eIF4E phosphorylation may streamline patient accrual in clinical trials utilizing these drugs.

Key words – cancer, eif4e, mtor, prostate, resistance

<<7>>

GENOMIC PROFILING OF NON-SMALL CELL LUNG CANCER PATIENT-DERIVED XENOGRAFT MODELS FOR PERSONALIZED CANCER THERAPY

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Background: Molecularly targeted therapy has changed the treatment paradigm for non-small cell lung cancer (NSCLC), which is the most common and lethal cancer worldwide. We previously reported that NSCLC patient-derived xenograft (PDX) models have good histomorphological and genotyping (EGFR and KRAS mutations) correlation with original patient's NSCLC tumors. The objective of this study is to expand the molecular characterization of the paired PDXs and original patient's NSCLC tumors to profile all currently "druggable" oncogene targets in the clinic.

Method: Genomic DNA from archival formalin-fixed, paraffin-embedded (FFPE) patient's tumors and fresh first human-to-mouse (P0) PDX tumors were isolated and subjected to Sequenom® oncogenotype mutation profiling using OncoCarta Panel v1. This panel detects 238 known mutations in 19 genes commonly altered in cancer.

Results: Our initial results show that in 7 of the 9 patient-PDX NSCLC models tested, the mutational status of oncogenes has been preserved. Two of the models had inconsistent genomic profiling results. It is as yet unclear if this is due to genomic instability or tumor heterogeneity.

Conclusion: Our results validate the overall genomic fidelity of P0 PDX tumors compared to original patient's tumors. However, molecular characterization is needed for each PDX model before using the model as a clinically relevant research platform for selecting and validating clinically relevant drug target(s) for personalized cancer therapy.

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<<8>>

REGULATION OF ONCOGENIC EZH2 BY ANCCA/ATAD2 AND ANDROGEN IN PROSTATE CANCER

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EZH2 is a histone methyltransferase and the enzymatic component of PRC2 complex that contains SUZ12, EED and RbAp46 for H3K27 methylation. EZH2 is overexpressed in many types of cancers including prostate cancers and its increased levels are strongly associated with poor clinical outcomes. High levels of EZH2 promote cancer cell proliferation, invasion and tumor metastasis through suppression of CDKN1C/p57, the CDKN2A gene locus, E-cadherin, RUNX3, and antagonists of signaling pathways. Several mechanisms have been described to account for de-regulated EZH2 expression in prostate cancer cells, which include the activation by ERG and c-Myc and the loss of micro-RNA-mediated silencing. However, how the expression of EZH2 is regulated in the normal prostate and tumors and whether EZH2 overexpression in prostate cancer involves other important regulators is unclear. By examining EZH2 and the novel chromatin regulator ANCCA expression in different developmental stages and hormonal milieus, we found that their expression is highly induced at early development of prostate and is strongly regulated by androgen. In androgen sensitive LNCaP cells, physiological concentrations of androgen stimulate expression of EZH2 and other PRC2 genes (SUZ12, and EED). However, in castration resistant prostate

cancer (CRPC) cell line C4-2B, androgen suppresses EZH2 expression. The induction of EZH2 by androgen could be mediated by androgen-induced ANCCA and involves E2F and histone H3K4me3 methylase MLL1 complex. Together, these results suggest the existence of an ANCCA-MLL-EZH2 chromatin regulator network in development of normal prostate and prostate cancer.

<<9>>

TREATMENT FOR REFRACTORY BRAIN METASTASES IN PATIENTS WITH EGFR MUTATION-POSITIVE LUNG CANCER

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The presence of *gain-of-function* mutations in the epidermal growth factor receptor (EGFR) tyrosine kinase domain defines a molecular cohort of non-small cell lung cancer (NSCLC) patients whose tumors respond to EGFR tyrosine kinase inhibitors (TKIs) erlotinib or gefitinib with a median survival of 21-31 months. However, the incidence of brain metastases, either at presentation or during the disease course, has been rising or is difficult to treat. Here, we describe a clinical course of 48-year old female, never smoker with recurrent intracranial and leptomeningeal brain metastases from lung adenocarcinoma harboring a common drug-sensitive EGFR mutation. The unique distribution of periventricular and leptomeningeal metastases and three brain recurrences despite of a good systemic control highlight the challenges and opportunities to develop new treatment strategies for this dreadful disease. We will also present early preclinical data demonstrating promising antitumor activity of nanoparticle based targeted therapy for the treatment of brain metastases.

<<10>>

STABILIZATION AND SEQUESTRATION OF THE UBIQUITIN LIGASE NRDP1 BY THE RETICULON RTN4A

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Dysregulation of the E3 ubiquitin ligase Nrdp1 has been implicated in a variety of diseases, including several cancer types. In breast cancer, for example, the post-translational loss of Nrdp1 is associated with increased levels of its substrate ErbB3, a potent receptor tyrosine kinase that contributes to tumor growth, progression and therapeutic resistance. Defining mechanisms of Nrdp1 regulation is therefore important for understanding its effects in normal development, where global loss of Nrdp1 is embryonic lethal, as well as in disease progression. Like many E3 ligases, Nrdp1 is a highly labile protein that undergoes autoubiquitination and degradation, and known mechanisms of post-translational control of Nrdp1 expression modify this autoubiquitination. Other mechanisms of Nrdp1 stabilization are likely to exist.

In this study we uncover a novel mechanism of Nrdp1 stabilization through its interaction with the reticulon Rtn4A. Rtn4A (Nogo-A) is best characterized as an inhibitor of axonal regeneration in the central nervous system, however, recent studies have shown that Rtn4A and the related reticulon proteins induce membrane curvature to form the endoplasmic reticulum (ER) tubule network. The Nrdp1-Rtn4A interaction sequesters Nrdp1 from a cytoplasmic/perinuclear distribution into ER tubules, resulting in stabilization of both Nrdp1 protein and the Nrdp1 substrate ErbB3. This sequestration may play a role in differentiation of neuromuscular junctions, as knockdown of Rtn4A in C2C12 myotubes destabilizes both Nrdp1 and ErbB3, which has been proposed as a regulator of neuromuscular junctions. In addition to a possible developmental role, Rtn4A-mediated stabilization of Nrdp1 may be associated with disease states, as Rtn4A expression in the brain could impact Nrdp1 function in diseases such as gliomas or Parkinson's.

<<11>>

REPRESSION OF CYCLIN G2 BLUNTS THE CELL CYCLE ARREST RESPONSE OF MCF-7 CELLS TO ANTAGONISTS OF ESTROGEN RECEPTOR SIGNALING AND THE AMPK ACTIVATOR METFORMIN.

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Acquired tumor cell resistance to endocrine-based therapeutics poses a significant challenge for long-term abatement of estrogen receptor (ER)-positive breast cancers (BCs). Signaling cross-talk between activated ER and peptide growth factor receptor pathways (e.g. HER2 and IGF-1R) is one adaptive mechanism promoting ER positive BC tumor cell resistance to drugs inhibiting estrogen (E2) signaling. Identification of the gene products regulated by these pathways that influence resistance to the ER-antagonists tamoxifen and fulvestrant could improve future therapeutic approaches for control of BC. Transcript levels of the *CCNG2* gene encoding the unconventional cell cycle arrest response protein, cyclin G2 (CycG2), are reduced upon activation of ER, HER2, insulin and insulin-like growth factor-1 receptor (IGF-1R) signaling. Our previous work showed that blockade of HER2, PI3K and mTOR signaling upregulates CycG2 expression in HER2 overexpressing BC cells, and that ectopic expression of CycG2 induces cell cycle arrest of BC cell lines. Here we show that E2 depletion and pharmacological blockade of ER signaling in E2-dependent BC cells enhances CycG2 expression and nuclear localization. Using shRNA-mediated RNAi we determined that blunting CycG2 upregulation promotes proliferation of E2-deprived and fulvestrant-treated MCF7 cells. Evidence suggests that loss of CycG2 increases phopho-activation of MEK1 and inhibitory phosphorylation of RB. Our work also indicates that CycG2 can form complexes with CDK10, a recently identified determinant of tamoxifen resistance linked to inhibition of the RAF/MEK/MAPK signaling pathway. Recent studies suggest that the anti-diabetic drug metformin (MTFN) inhibits BC cell growth by promoting AMPK–mediated suppression of growth factor receptor activation of mTOR. Patients taking MTFN exhibit a dose-dependent reduction in cancer risk and clinical trials indicate that BC therapies including MTFN improve response rates. We found that MTFN treatment stimulates CycG2 expression and potentiates both fulvestrant-mediated upregulation of CycG2 expression and growth-inhibition of MCF-7 cells. Moreover knockdown of CycG2 expression blunts the enhanced anti-proliferative effect of MTFN on fulvestrant treated cells. Importantly, analysis of BC tumor cDNA microarray databases indicates that *CCNG2* transcripts are reduced in aggressive, poor-prognosis BCs.

<<12>>

ERBB3 OVEREXPRESSION INDUCES RESISTANCE TO DUAL EGFR/ERBB2 INHIBITORS IN PROSTATE CANCER

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Androgen withdrawal therapy (AWT) remains standard therapy for advanced prostate cancer (PCa) but patients eventually relapse into castration resistant PCa (CRPC). Two major members of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases (RTK), ErbB2/HER2 and ErbB3/HER3, are overexpressed and contribute to CRPC progression. We previously showed that dual inhibition of EGFR and HER2 prevented activation of ErbB3. Hence we investigated whether the reversible, dual-kinase HER2/EGFR inhibitor lapatinib ('Tykerb') prevented PCa progression and prolonged sensitivity to AWT. Lapatinib inhibited the growth of androgen-dependent but not CRPC cell lines. Surprisingly, androgen-dependent CWR22 tumour xenografts grew better in lapatinib when androgens were present. EGFR was significantly phosphorylated at Y1068 in lapatinib-resistant lines but knockdown (siRNA) prevented the growth of lapatinib-resistant CWR-R1 cells. Therefore we investigated dacomitinib (PF00299804, Pfizer), a dual EGFR/ErbB2 inhibitor primarily targeting EGFR. Dacomitinib suppressed EGFR phosphorylation at Y1068 and was singularly effective in preventing ErbB3 phosphorylation at Y1289 despite not directly binding to it. ErbB3 overexpression induced dacomitinib resistance but this was overcome by suppressing androgen signaling. These preclinical data indicate that cells overexpressing EGFR or ErbB3 are resistant to dual EGFR/ErbB2 inhibitors so preselection of patients with low ErbB activation may demonstrate greater efficacy of ErbB inhibitors in future clinical trials.

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<<13>>

COMPARISON OF THE EFFECTS OF FOUR ERBB INHIBITORS IN TWO BLADDER CANCER CELL LINES EXPRESSING HIGH AND LOW LEVELS OF THESE RECEPTORS

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Background: Various studies demonstrated increased expression of EGF-receptor (EGFR/HER1/ErbB1) and related receptors ErbB2/HER2/Neu, ErbB3/HER3 and ErbB4/HER4 in bladder cancer. Clinical trials using drugs inhibiting EGFR (erlotinib), ErbB2 (trastuzumab) or dual EGFR/ErbB2 inhibitors (lapatinib) were all deemed ineffective. We hypothesized that inhibition of one receptor causes increased expression of the others meaning all four must be suppressed to achieve lasting effects. Here we compare the effects of these drugs in two bladder cancer cell lines to a novel pan-ErbB inhibitor dacomitinib.

Methods: The bladder cancer cell lines, T24 and TCCSUP, were used to evaluate the efficacy of erlotinib (Tarceva), trastuzumab (Herceptin), Tykerb (Lapatinib) and Dacomitinib (PF00299804). Cell growth was estimated by MTT or crystal-violet staining. Protein expression was determined by western blotting and apoptosis was determined by flow cytometry after seventy-two hour incubation with the indicated treatment.

Results: Comparison of T24 and TCCSUP bladder cancer cell lines with C4-2 (prostate) and MCF-7 (breast) cell lines showed that bladder cells expressed high levels of EGFR and ErbB3 but not ErbB2, while TCCSUP cells expressed higher levels of ErbBs than T24. Interestingly, ErbB2 and ErbB3 are readily activated in T24 cells compared to TCCSUP. MTT demonstrated the efficacy of dacomitinib and erlotinib, primarily EGFR inhibitors, compared to lapatinib, primarily an ErbB2 inhibitor with some anti-EGFR activity, while the ErbB2 inhibitor, trastuzumab, had little effect. The trastuzumab and erlotinib combination performed worse than erlotinib alone in T24 cells; hence trastuzumab studies were not pursued. Crystal-violet staining after treatment with erlotinib, lapatinib and dacomitinib indicated higher efficacy of dacomitinib after seven days. MTT and flow cytometry showed that lapatinib was ineffective alone, but in combination with dacomitinib significantly decreased cell viability in TCCSUP cells. In contrast, combining all three drugs proved most effective in T24 cells.

Conclusions: Higher expression of the ErbB receptors in TCCSUP cells likely result in greater sensitivity to inhibitors of the EGFR family. Therefore, evaluation of the levels of these receptors would yield better response to ErbB inhibitors.

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<<14>>

THE ANDROGEN RECEPTOR MODULATES P14ARF EXPRESSION IN HUMAN PROSTATE TUMOR AND TUMOR DERIVED CELL LINES

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The p53 tumor suppressor pathway is essential for the maintenance of genomic integrity and is frequently altered in tumor cells, but its regulation is modulated by multiple factors. MDM2 and MDM4 target p53 for degradation. The tumor suppressor p14arf, can negatively regulate MDM2, thus should increase p53 levels and inhibit tumor formation. However, the exact opposite has been described in prostate cancers- p14arf levels increase with tumor stage and grade. affecting p53 levels. Thus far, studies have not analyzed the expression of p53, MDM2, MDM4, AR and p14arf in human prostate tumors. We used archival prostate

tumor tissues obtained from prostatectomies performed at the Veterans Affairs-Northern California Health Care System in Mather California between 1996 and 2002. A prostate tumor tissue array consisting of 78 tumors used to evaluate possible correlations among multiple parameters: stage, surgical Gleason grade, pre-operative PSA levels and race. Immunohistochemical studies assessed expression of the proliferation marker Ki67, tumor suppressor p53, p53 regulators MDM2 and MDM4, and the AR and p14arf. p53, MDM4, p14arf and AR is expression is predominantly nuclear in tumor and adjacent cells, while in tumor cells there is a shift to a more cytoplasmic MDM2 expression. There was an increase in the cytoplasmic expression of MDM2 and nuclear expression of MDM4, p14arf and AR in tumor tissue. Multivariate analysis identified a strong correlation between expression of p14arf and AR. Additional studies expanded on this observation and revealed that in prostate tumor-derived cells siRNA-mediated AR ablation in resulted in decreased transcription of p14arf. Studies of AR and p14arf expression in the castrate sensitive CWR22 xenograft model also showed a correlation between the expression of AR and p14arf, where expression of both is decreased following castration. This study argues that the AR positively regulates the expression of p14arf in in human prostate tumors.

<<15>>

ANDROGEN RECEPTOR-MEDIATED REGULATION OF NON-CODING RNAS IN TWO CASTRATE RESISTANT CELLS LINES- AN ADDITIONAL LAYER OF COMPLEXITY

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The central dogma that DNA is transcribed into RNA, and translated into proteins, has been challenged by the discovery that large arrays of transcripts are not translated. The function of small RNAs has been better defined, but the role of the majority of longer transcripts in cell physiology is not well understood. Several different functions have been ascribed to these RNAs: 1) scaffolding to bring several proteins into spatial proximity 2) assistance in chromosome looping, 3) decoys that titrate away regulatory molecules, and 4) guides that through RNA-DNA interaction recruit DNA binding proteins to regulatory regions. Since these RNAs have been implicated in the regulation of cell cycle and stem cell pluripotency, we used RNA-seq to identify transcripts that are androgen receptor (AR) regulated in two castrate resistant, AR-dependent cell lines- 22Rv1 and LNCaP-AI. Similarly to protein coding transcripts, some of the non-coding RNAs are AR regulated in only one line, while others are AR regulated in both. Our analysis found that ncRAN,a long non-coding transcript that has been associated with bladder cancer is expressed and AR regulated. Transcripts that map to the MAP3K14 intronic region, which has been implicated in pancreatic cancer, are AR repressed in both cell lines. The ZNF300 pseudogene ZNF300P1 transcript is AR regulated in 22Rv1 as is the ZNF300 transcript, indicating that the two are coordinately expressed. Several snoRNA host genes (SNHG1, 3 and 4), whose function is unknown, are AR regulated as well. Finally we noted that there was abundant expression and AR regulation of transcripts encoded by genomic regions that have no annotated protein coding or non-coding sequences. This study further expands the repertoire of AR-dependent genes that may have a role on prostate cancer biology.

<<16>>

SYNTHESIS OF MARTINELLINE AND MARTINELLIC ACID: POTENTIAL INHIBITORS OF COLON CANCER CELL PROLIFERATION

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Colon cancer is the leading gastrointestinal (GI) cause of death in the United States. Fortunately, detecting colon cancer in its early stages allows the use of treatment with great outcome. However, once the cancer becomes invasive, the effectiveness of current therapies greatly diminishes. Therefore, there is a need to develop new therapeutic compounds that can halt the proliferation of colon cancer cells. In 1995, Varga et. al. published the isolation of martinellic acid and martinelline from an organic extract of *Martinella quitosensis* roots. Of these two compounds, the latter has shown an affinity for the muscarinic receptor M_3 ($CHRM_3$). Muscarinic receptors play an important role in the stimulation and proliferation of human colon cancer cells. It has been reported that the deficiency of the muscarinic receptor $CHRM_3$ reduces intestinal neoplasia. We hypothesize that due to the high affinity of martinelline for $CHRM_3$ (K_b= 0.1mM), this natural

product can be used to study the muscarinic receptor signaling pathway and potentially lead to a new therapeutic compound for the treatment of colon cancer. Methodology that has been developed in our lab, imine-anhydride lactam formation, will facilitate the synthesis of martinelline. Once synthesized, tests will be conducted to determine which functional groups are responsible for its biological activity.

<<17>>

INDUCTION OF RAPID TELOMERE SHORTENING BY ELEVATED LEVELS OF TRF2

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Telomeres are specialized nucleoprotein structures at the ends of linear chromosomes. Mammalian telomeres consist of TTAGGG tandem repeats bound by Shelterin protein complexes. Telomeric Repeat Binding Factor 2 (TRF2) is the double stranded telomeric binding protein essential for protecting the integrity of telomeres. TRF2 overexpression has been found to lead to rapid and drastic telomere shortening in mammalian cells. However, the molecular mechanisms behind the TRF2-induced telomere shortening are unclear. Our data shows that telomere shortening induced by elevated levels of TRF2 in human cells results in chromosome end-to-end fusions. And the majority of the fusions do not have detectable telomere signals at the fusion junction, indicating a complete loss of telomeric repeats on these chromosomes. Immediately upon TRF2 overexpression, often several cell divisions before significant amounts of telomere shortening or chromosome end-to-end fusions can be detected, many extremely fine telomere bridges are formed between segregating chromosomes during anaphase, suggesting that these telomere bridges are not caused by chromosome fusions. We are currently investigating the hypothesis that elevated levels of TRF2 induces replication fork stalling and incomplete sister telomere segregation.

TRF2 protein levels are reported to be much higher in many tumor tissues compared to normal tissues. Furthermore, during immortalization of human mammary epithelial cells (HMECs), TRF2 protein levels increased 10-15 fold, suggesting that elevated levels of TRF2 might promote immortalization/transformation of primary cells. Our study of TRF2-induced telomere shortening may provide insight on how elevated levels of TRF2 facilitate cancerous transformation.

<<18>>

HIGHER BASELINE TNF RECEPTOR AND TNF-A EXPRESSION IN MACROPHAGES OF AGED MICE

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Recent studies by our lab have shown that aged mice are much more susceptible to toxicity affecting multiple organs caused by cancer immune stimulatory therapies. We have recently found that this toxicity is dependent upon TNF-α secreted by macrophages. This toxicity after immunotherapy is exacerbated in animals with higher fat content. We hypothesized that this may be due to up-regulation in baseline levels of TNF receptors. To test our hypothesis, we compared bone marrow derived macrophages from young (4 months), middle aged (10 months), and aged (20 months) mice and examined baseline expression of TNFR1α, TNFR1β, and TNFα by qPCR. At baseline, there were no differences between young and middle aged (4 and 10 months respectively) mice. However, aged (20 months) mice had significantly increased expression of TNFR1α ($p<0.01$), TNFR1β ($p<0.01$), and TNFα ($p<0.05$) compared with both young and middle aged mice. These results suggest that higher receptor expression may explain the heightened TNFα associated toxicity seen in aged mice. Further analysis of different tissues including adipose tissue as well as other parenchymal tissues may shed insights on this heightened pro-inflammatory response during aging.

<<19>>

N-GLYCAN PROFILES AS POTENTIAL MARKERS FOR THE EARLY DETECTION OF LUNG CANCER

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Lung cancer is the leading cause of cancer death for men and women in the United States. Currently there are no reliable biomarkers able to identify early stage disease and no FDA- approved serum test. The most prevalent form is Non-small cell lung cancer (NSCLC), which, if diagnosed early has a very good five-year survival rate. N-glycans are oligosaccharide chains attached to proteins, and the overall glycan profile has been shown to be altered in plasma of patients suffering a diverse range of cancer, including ovarian, breast, pancreatic and lung cancers. However, such studies have all be performed using plasma from late-stage cancer patients, which cannot yet be extended towards pre-diagnostic early stages of lung cancer. In this study, N-glycan profiles were obtained from 299 serum samples from the CARET study, of which 199 controls and 100 pre-diagnostic lung cancer patients. The cohort was adjusted for age, sex and smoking history. N-glycan profiles were obtained using a mass spectrometric method, based on chip-based nanoLC coupled to time-of-flight mass spectrometry. The stability of the analytical platform was thoroughly tested, and was shown to be highly repeatable, thus providing a suitable form of analysis for larger-size cohorts. Using statistical analysis, several glycans could be identified that were altered pre-diagnostically with lung cancer.

Overall, we here present the development of glycan based candidate biomarkers for the early detection of lung cancer from serum using a robust and rapid throughput platform.

<<20>>

TUMOR INOCULATION WITH E. AEROGENES RESULTS IN PROLONGED SURVIVAL IN A RAT GLIOBLASTOMA MODEL: INITIAL PROOF OF CONCEPT FOR INTRACRANIAL PROBIOTIC THERAPY.

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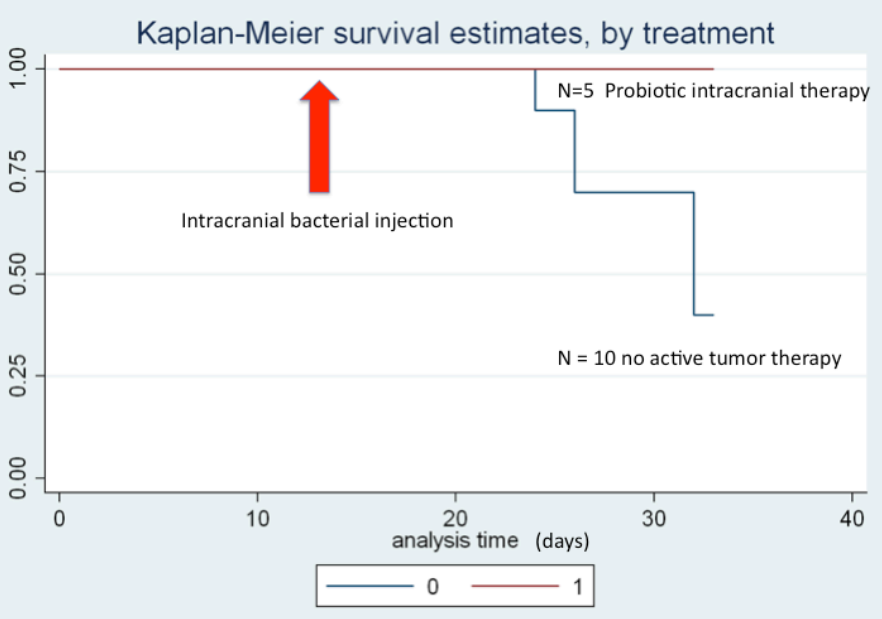
Introduction: Wound infection may prolong survival in glioblastoma. Possible mechanisms include immunomodulation or direct bacterial oncolysis. We developed a platform for delivering immobilized bacteria directly to the tumor bed through stereotaxic inoculation. We tested this therapy in the immunocompetent syngeneic rat glioblastoma model Lewis/CNS-1.

Methods: Enterobacter aerogenes primary cultures (ATCC 13048) were prepared by a water-in-oil emulsion method to produce bacteria-laden 2% agarose microbeads. CNS-1 tumors were seeded into the caudate of 12-week old (285-300g) Lewis male rats by stereotaxic injection of $1.25x10^3$ cells via Hamilton syringe (0.0 mm anterior-posterior and 3.0 mm right lateral to bregma, 5.0 mm subdural). Two weeks later, rats were injected with 10 µL microbeads (40-100 µm diameter) at the same coordinates. We used 6 experimental and control groups with 5 animals/group. Experimental and control groups were as follows: **A** untreated tumor, **B** tumor + sterile microbeads, **C** tumor + E. aerogenes microbeads, **D** E. aerogenes microbeads only, **E** sterile microbeads only, **F** sham surgery. Survival data was collected for up to 5 weeks.

Results: 2 animals died immediately following surgery of procedural complications (one each in groups **B** and **E**). All other animals survived up to 3 weeks without any other therapy. By week 4, three out of five of the animals with tumor only had died (Group **A)**. By week 5, 2 out of the 4 remaining animals with tumor and sterile microbeads had died (Group **B**). Kaplan-Meyer survival curves between treated and untreated tumor groups were significantly different at 33 days ($p=0.0393$, log-rank test for equality of survivor function).

Conclusions: This preliminary data suggests that intracranial E. aerogenes inoculation significantly reduces the risk of tumor death in an animal model of glioblastoma. Further research is needed to confirm this effect in other animal models and to investigate the mechanism of action.



<<21>>

THE EFFECTS OF MM-121 ON ANDROGEN DEPENDENT AND INDEPENDENT PROSTATE CANCER CELLS

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Prostate cancer remains a very prevalent form of cancer among men. Androgen withdrawal therapy is the standard-of-care, but some patients develop castration-resistant prostate cancer and no longer respond to androgen withdrawal. Upregulation of the tyrosine kinase EGFR family and cell signaling through ErbB3 activation facilitates proliferation after androgen withdrawal. Subsequently, the monoclonal antibody, MM-121, an ErbB3-specific inhibitor for the ligand binding domain, was used to study the effects of ErbB3 inhibition on prostate cancer growth in media with and without androgens. Cell proliferation assays were used to study how varying concentrations of MM-121 affect cell growth in LNCaP (androgen-dependent) and PC-3 (androgen-independent) cell lines. Its effects on relative protein levels of the EGFR family were tested on LNCAP cells. Our research demonstrates that MM-121 treatment results in biphasic activation of proliferation pathways: proliferation increases sharply at lower concentrations, sharply drops at medium levels, and increases again at high concentrations. Correspondingly, protein levels demonstrated that at high concentrations, but not at medium or low levels, MM-121 stimulated an increase in EGFR and a decrease in AR. Surprisingly, higher doses of MM-121 suppressed ErbB3 phosphorylation when stimulated by heregulin (HRG) but not with EGF, suggesting that MM-121 causes EGFR to phosphorylate ErbB3 through dimerization and not ligand binding. These results indicate that inhibition of ErbB3 alone would not prevent growth and survival of prostate cancer cells.

Acknowledgements: This work was supported partially by a donation of the drug MM-121 and funds from Merrimack, Inc, and a grant from the Department of Defense.

<<22>>

UTILIZATION OF TRANSCRIPTOME SEQUENCING FOR THE IDENTIFICATION OF BLOOD-BASED BIOMARKER SIGNATURES OF HEPATOCELLULAR CARCINOMA IN HBV-INFECTED ASIAN AMERICANS.

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Hepatocellular carcinoma (HCC) has emerged as the cancer site with the greatest annual increase in mortality rates in the United States. Chronic hepatitis B virus (HBV) infection is the major etiological factor with carriers having a 100-fold greater risk of developing HCC than uninfected individuals. Asian Americans are significantly impacted since HBV infection is 30-75 times more prevalent than in the general U.S. population and 80% of liver cancers in Asian Americans are HBV-associated. The overall goal of our research is to utilize next-generation sequencing (NGS)-based transcriptome profiling in order to identify blood expression profiles that could serve as biomarkers for early detection, diagnosis, and surveillance of HCC. In this pilot study, RNA-Seq was performed on total RNA isolated from whole blood samples obtained from three HBV-positive, Asian American patients, one of which had HCC. Paired-end libraries were sequenced (2 x 40-bp) with an Illumina GAIIx and our standard analysis pipeline was used for processing raw sequence data, sequence alignment, SNV detection, and transcript assembly, quantitation, and differential expression analysis. Whole blood peripheral blood mononuclear cells from the HCC patient possessed a distinctive expression signature defined by 634 differentially expressed genes. Meta-analysis of the 173-gene overexpression cluster with immune cell transcriptome data (GSE3982) revealed that this "HCC signature" contained prominent neutrophil- and macrophage-driven components. Gene ontology analysis indicated over-represented processes consistent with HCC pathogenesis, including inflammation. Finally, the superb sensitivity and versatility of this approach was underscored by detection of low-level HBV viral gene transcription. In closing, these results demonstrate that the presence of HCC and extrahepatic HBV infection can be detected by analyzing blood transcriptional profiles. Future studies will be directed at evaluating the role of NGS-based blood genomics in the early detection of HCC.

Supported by grants U54CA153499, U54CA153499-02S1, P01CA109091-A1, and Office of Research, UCD School of Medicine, and Cancer Center Support Grant P30 CA93373-01.

<<23>>

A NEW FAMILY OF PLURIPOTENCY-RELATED ONCOGENES

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There are many important links between tumorigenesis and pluripotency. For example, the genes used to produce induced pluripotent stem cells (IPSC) and those most highly expressed in human embryonic stem cells (hESC) are strongly associated with cancer. In fact, one of the most troubling roadblocks to stem cell-based therapies is that both hESCs and IPSCs possess innate tumorigenicity. At the same time understanding the role of pluripotency machinery in cancer will open doors to new treatments based on targeting cancer stem cells.To search for stem cell-related oncogenes that might be important in cancer, an expression screen for novel pluripotency-related oncogenes using a retroviral ESC cDNA library was conducted. From this screen, we identified not only known oncogenes but also intriguingly the key pluripotency factor, DPPA4 (developmental pluripotency-associated 4). In direct transformation assays, we validated that DPPA4 is an oncogene in both mouse 3T3 cells and immortalized human dermal fibroblasts (HDFs). Overexpression of DPPA4 generates oncogenic foci (sarcoma cells), causes anchorage-independent growth, and gives rise to tumors in immuno-deficient mice. Furthermore, functional analysis results indicate that both the DNA-binding SAP domain and the histone-binding C-terminal domain are critical to the oncogenic transformation activity of mDPPA4. In addition, charaterizations and gene expression studies of the DPPA4-tranformed cells suggest that DPPA4 can induce cell proliferation through genes related to regulation of G1/S transition. Interestingly, we observed similar findings for family member DPPA2. Thus, we have identified a new family of pluripotency-related oncogenes consisting of DPPA2 and DPPA4. Our findings have critical implications for understanding stem cell biology and tumorigenesis. They also provide a foundation for the development of new cancer treatments and safer approaches to stem cell-based regenerative medicine.

<<24>>

TRANSDUCTION AND TRANSPLANTATION OF MAMMARY EPITHELIAL CELLS TO EXPLORE NRDP1 FUNCTION

Jessica Wald, Jason Hatakeyama, David Boucher, Colleen Sweeney, Russell Hovey, & Kermit Carraway, III

ErbB3/HER3 is a receptor tyrosine kinase that contributes to the proper development of a variety of tissues, and its overexpression and aberrant activation in tumors contributes to breast cancer initiation, progression and therapeutic resistance. Nrdp1, an E3 ubiquitin ligase, binds to and regulates ErbB3 levels through ubiquitination and degradation of excess receptor in normal tissue. Nrdp1 is found at reduced amounts in over 70% of primary human breast tumors that overexpress ErbB3, suggesting that Nrdp1 may play an important role as a tumor suppressor protein. Epithelial cells that line the mammary duct display an asymmetric organization or "polarity" that is essential for appropriate mammary gland formation and function. Disruptions in cell polarity disrupt mammary gland organization and outgrowth, and this loss of polarity is characteristic of high grade breast cancers and poor prognosis. Importantly, Nrdp1 also interacts with the known polarity proteins, MARK4 and Vangl1/2, suggesting roles in the establishment or maintenance of epithelial cell polarity. However, the direct role of Nrdp1 in mammary gland biology and breast cancer development has not yet been elucidated.

To study Nrdp1's role in mammary gland development and breast cancer, our lab has developed a transduction/ transplantation protocol to overexpress Dominant Negative-Nrdp1 (DN-Nrdp1) in the mammary gland. Primary mouse mammary epithelial cells (mMECS) expressing the control mCherry marker grew out 8 weeks after transplantation into a cleared (epithelial-free) mammary fat pad, forming normal epithelial ductal structures. Transplanted mMECs expressing DN-Nrdp1 in addition to the mCherry marker, however, failed to grow out and form mammary ducts and acini, indicating that Nrdp1 plays a crucial role in mammary development.

<<25>>

HISTONE METHYLASE NSD2 DRIVES ENDOCRINE THERAPY RESISTANCE BY STIMULATION OF MULTIPLE KINASE SIGNALING PATHWAYS

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Endocrine therapies targeting estrogen action (e.g. tamoxifen and aromatase inhibitors) are effective in decreasing mortality from breast cancer (BCa). However, their efficacy is limited by intrinsic and acquired resistance. Development of specific biomarkers for better prediction of therapy response and identification of new therapeutic targets for the resistant disease remain major challenges. Multiple resistance mechanisms involving deregulation of several cellular pathways have been proposed. However, how the molecular mechanism underlying the deregulation is poorly understood. We performed immunohistochemistry (IHC) analysis of a cohort of BCa tumors for histone methyltransferase protein NSD2 (a.k.a. MMSET) and found that NSD2 was overexpressed in over 30% of ER-positive tumors. Further study suggests that high levels of NSD2 are associated with poor survival of patients who received tamoxifen treatment. Remarkably, NSD2 overexpression in tamoxifen-sensitive BCa cells stimulates their estrogen-independent proliferation and resistance to 4-hydroxy-tamoxifen. NSD2 overexpression also confers resistance to tamoxifen treatment in xenograft tumors. Mechanistic studies demonstrated that elevated NSD2 directly stimulated the expression of key receptor tyrosine kinases including EGFR and HER2 and the activation of PI3K/Akt and mTOR pathways. Further analysis revealed that aberrant NSD2, through its histone methylase, activated the estrogen related receptor-alpha (ERRa) function to impinge on the ERa signaling. Together, our findings suggest that aberrant expression and function of epigenetic regulators such as NSD2 can drive endocrine therapy resistance and that small molecules targeting histone methylases such as NSD2 can be effective in treatment of therapy-resistant BCa tumors.

<<26>>

INHIBITING HEDGEHOG SIGNALING ACTIVITY SUPPRESSED THE TUMORIGENICITY, PROLIFERATION AND METASTASIS OF HEPATOMA SUBPOPULATIONS

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BACKGROUND: In a previous study we found that most poorly-differentiated hapatoma cells are CD133/EpCAM-negative, whereas they are more chemoresistant and metastatic, and have a higher level of hedgehog (Hh) signaling activity than $CD133^+/EpCAM^+$ cells, which are more tumorigenic (J Hepatol 2011; 55: 838-845). The aim of the present study was to investigate the effects of an Hh signaling inhibitor, LDE225, a SMO antagonist, on cell proliferation, tumorigenicity and metastasis of hepatoma subpopulations.

METHODS: Huh-7 and HLF cells were separated by FACS enrichment into $CD133^+/EpCAM^+$ or $CD133^-/EpCAM^-$ subpopulations, and their tumorigenicity, proliferation and metastasis were evaluated by spheroid formation, WST-1 assay and Matrigel invasion assay, respectively.

RESULTS: $CD133^+/EpCAM^+$ Huh-7 cells were E-cadherin-positive; but Zeb1- and GLI2-negative by immunocytochemistry. In contrast, the $CD133^-/EpCAM^-$ Huh-7 cells were E-cadherin-negative; but Zeb1- and GLI2-positive, and so were the $HLF^-/^-$ cells. Transfection with a Gli2-Luc reporter plasmid confirmed much more enhanced Hh signaling activation in double negative cells than double positive huh-7 cells. The treatment with LDE225 at 20-100 nM significantly suppressed the number and size of spheroids from $CD133^+/EpCAM^+$ Huh-7 cells compared to untreated controls ($p<0.05$-0.01). Treatment with LDE225 at 20 nM significantly inhibited proliferation of $Huh\text{-}7^-/^-$ cells ($p<0.05$), but was not effective for $Huh\text{-}7^+/^+$cells. $Huh\text{-}7^-/^-$ cells displayed a higher invasion rate than the $Huh^+/^+$ counterpart, and the invasion was suppressed by LDE225 at 20nM in both subpopulations.

CONCLUSIONS: Poorly-differentiated hepatoma cells are $CD133^-/EpCAM^-$, and they exhibited profound epithelial mesenchymal transition and enhanced hedgehog signaling activation. These changes may contribute to their enhanced metastasis. Blocking Hh signaling activity by inhibiting the SMO molecule with LDE225 at nanomolar levels could effectively suppressed the proliferation, tumorigenicity and metastasis of the double-negative subpopulation, which otherwise is chemo-resistant. This observation underscores the significance of targeting hedgehog signaling molecules as a novel therapy for poorly-differentiated hepatocellular carcinomas.

<<27>>

SYNERGISTIC COMBINATION OF MICRONEEDLES AND BIOCHEMICAL APPROACHES FOR INTRA-TISSUE DELIVERY OF OLIGONUCLEOTIDES IN LIVING CELLS IN 3D TISSUE PHANTOMS

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Delivery of oligonucleotides (ONs) to target cells in-vivo can lead to significant therapeutic and diagnostic applications to cancer such as treatment of cancer using siRNAs and detection of diseased states at the RNA level. Despite many developments, significant challenges in delivery of ONs to target tissues remain. These challenges include efficient distribution of ONs over a large tissue area and across the thickness of tissues, efficient internalization of ONs in cells in 3D tissues and avoidance of damage to cells as well as patients during delivery process. In this study, a minimally invasive approach for localized intracellular delivery of ONs in 3D model tissues was developed and evaluated. This approach is based on a synergistic combination of micron-scale needle arrays (microneedle array) and biochemical agents for promoting intra-cellular delivery. Two biochemical approaches were evaluated: co-delivery of ONs and streptolysin O (SLO) as well as cholesterol-conjugated ONs. Intratissue and intracellular distributions of ONs in 3D model tissues were characterized using optical imaging. Results show that ONs can be uniformly coated on microneedles and efficiently released within 5 minutes after insertion. ONs can be efficiently delivered to a lateral distance of 300-400 microns from the microneedle insertion point in a tissue model within 35 minutes of incubation using SLO, and more than 500 microns while cholesterol conjugated ONs were used. Results also show that ONs can be delivered across the thickness of the tissue (~500 microns). Both biochemical approaches demonstrate high cellular penetration in delivering of ONs in 3D tissues. For functional delivery, cholesterol-conjugated antisense ONs targeting EGFP demonstrate partial but significant reduction in EGFP expression. Overall this study shows that microneedles and SLO or cholesterol conjugation of ONs can result in widespread delivery of ONs into cells of 3D tissues.

<<28>>

ROLES OF NON-PROTEINACEOUS LIPID MEDIATORS IN ANGIOGENESIS

Zhang Guodong et al.

The formation of new blood vessels (angiogenesis) is vital for tumor growth and metastasis. While most effort to elucidate the angiogenic mediators focus on proteins, the roles of non-proteinaceous lipid mediators have received little attention. Epoxyeicosatrienoic acids (EETs) and epoxydocosapentaenoic acids (EDPs) are lipid mediators produced by cytochrome P450 (CYP) epoxygenases from omega-6 arachidonic acid (ARA) and omega-3 docosahexaenoic acid (DHA) respectively, and they both regulate inflammation and vascular tone. Here we show that EETs and EDPs have opposite actions on angiogenesis, tumor growth and metastasis. EDPs inhibited vascular endothelial growth factor (VEGF)- and fibroblast growth factor-2 (FGF-2)-induced angiogenesis *in vivo*. Systematical administration of 0.05 mg/kg/day EDPs, in conjugation with a low-dose pharmacological inhibitor of soluble epoxide hydrolase (sEH) to stabilize EDPs in circulation, caused ~70% reduction of primary tumor growth and metastasis. Contrary to the effects of EDPs, EETs increased tumor progression, which was consistent with a previous report. Together, these results demonstrate EETs and EDPs as novel endogenous regulators of angiogenesis and tumorigenesis, and suggest a potential mechanistic basis for the connection between omega-3 and omega-6 fatty acids and cancer.

FRIDAY POSTER PRESENTATIONS (ABSTRACTS)

<<1>>

ROLE OF PPARα IN PROLIFERATION AND CELL CYCLE REGULATION IN HUMAN RENAL CELL CARCINOMA

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The peroxisome proliferator-activated receptor (PPARα) is a ligand-activated transcription factor that belongs to the nuclear hormone receptor superfamily. PPAR*α* is expressed primarily in the liver, kidney, small intestine, heart, and muscle. The number of direct PPARα-target genes is large, but include many that encode enzymes that are involved in glucose, lipid and amino acid metabolism. Our metabolomics studies in human and mouse xenograft models of renal cell carcinoma (RCC) showed alteration in PPARα pathway metabolites in tissue, serum and urine (Ganti et al Cancer Research, 2012). While PPARα mediates hepatocarcinogenesis induced by long-term administration of PPARα agonists in rodent models the role of PPARα in renal cell carcinoma in human is not known. In this study we validate the role of PPARα *in vitro* by investigating its role in proliferation and cell cycle regulation on some RCC cell lines and normal proximal tubular epithelial cells of human kidney. When added to five different cell lines (three kidney cancer derived and two normal), the specific PPARα ligand, WY-14643, induced proliferation but the specific PPARα antagonist, GW6471, had the opposite effect as measured by MTT assay. Because cell cycle analysis showed a G0G1 cell cycle arrest in PPARα antagonist treatment groups, we evaluated expression levels of the cell cycle proteins (c-Myc, Cyclin D1, CDK4, CDK2). In the antagonist treatment groups, we found significant decrease in all of these proteins in time dependent manner and corresponding to the cell line genotype (VHL- or VHL+). These data revealed for the first time the involvement of PPARα in regulation of the cell cycle in human renal cells and that blocking PPARα might be a potential therapeutic target for RCC treatment.

<<2>>

IS MXD3 AN ONCOGENE?

Angel Alvarez, Gustavo Barisone and Elva Diaz

During development, Sonic hedgehog (Shh) regulates the proliferation of cerebellar granule neuron precursors (GNP's) in part via expression of Nmyc. Mutations in the Shh signaling pathway lead to brain tumors in mice and humans. Current models suggest that MXD3, a member of the Mad family of proteins, antagonizes Myc proteins by competing for the cofactor Max. This would predict that MXD3 could potentially suppress tumor progression. However, our lab has shown that MXD3 expression is upregulated in mouse models of medulloblastoma, the most common brain tumor in children. This result suggests that MXD3 may play a different role than originally hypothesized, acting as an oncogene rather than a tumor suppressor. To test the hypothesis that MXD3 overexpression could lead to tumorigenesis, we will perform oncogenic foci formation assays to assess MXD3 tumorigenic potential. Here we present the construction of retroviral tools to perform these assays, as well as the preliminary results obtained with these tools.

<<3>>

COMBINATION OF IL-2 AND ANTI-TGF- B INCREASES NATURAL KILLER CELL RECONSTITUTION AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Despite the efficacy of hematopoietic stem cell transplantation (HSCT) as a cancer therapy, cancer relapse remains a significant complication. Natural killer (NK) cells are the first lymphoid population to recover after HSCT and can kill transformed or virally-infected cells without prior sensitization. Therefore, accelerating NK cell reconstitution may improve HSCT therapy. We utilized the combination of administering the stimulatory cytokine, IL-2 concurrently with the blockade of the immunosuppressive cytokine, transforming

growth factor-beta (TGF-β) as a possible therapy to accelerate NK cell reconstitution after syngeneic HSCT. Resting C57BL/6 mice were treated with daily doses of IL-2 ($2x10^5$ IU) and anti-TGF-β (120ug, clone 1D11) every other day for one week. Although combinatorial therapy did not significantly expand NK cell numbers in comparison with IL-2 monotherapy, NK cytotoxicity, as measured by the ability to lyse Yac-1 tumor cells, was significantly greater. However in a HSCT model, combinatorial therapy administered in C57BL/6 mice starting at day 11 post-HSCT did significantly increase both the percentage of activated NK cells and NK tumor lytic ability compared with IL-2 alone. Additionally, mice showed no outward evidence of toxicity during administration of therapy. Together, these results suggest that this combination regimen improves reconstitution of NK cells after HSCT which may lead to better anti-viral and anti-tumor responses.

Grant Support: NIH RO1 HL089905.

<<4>>

NK CELLS MEDIATE PREFERENTIAL KILLING OF GLIOBLASTOMA CANCER STEM CELLS

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The cancer stem cell (CSC) hypothesis represents a paradigm-shifting concept in tumor therapy resistance. This small population of tumor initiating cells is relatively quiescent and therefore resistant to conventional cytoreductive cancer therapies which rely on DNA damage. It is these cells which may provide a major mechanism for cancer relapse and therapeutic resistance. We hypothesized that immunotherapy with activated NK cells will show greater efficacy in targeting CSCs than other chemo- or radiotherapies. This may be due to the presence of adequate levels of surface activating receptors for NK cell recognition of CSCs, whereas these cells may evade cytoreductive therapies due to their quiescent nature as well as high levels of drug efflux and DNA repair mechanisms.

Human NK cells were isolated from PBMCs and activated for up to 2 weeks with irradiated feeder cells. NK cells were then co-incubated with glioblastoma (GBM) or breast cancer (BC) cell lines for 18 hours then analyzed by flow cytometry for CSC markers on the surface of tumor cells. Immunocompromised mice were then implanted with orthotopic human glioblastoma xenografts and treated with activated NK cells in the presence or absence of homeostatic cytokines. Tumors were monitored for growth by bioluminescent imaging.

Our results demonstrated that high doses of irradiation isolated the CSC population within *in vitro* human cell lines in accordance with previous studies. However, overnight co-incubations with NK cells resulted in the CSC population to become preferentially targeted. When human NK cells were administered intracranially to orthotopic glioblastoma-bearing mice, tumors reduced in size or remained stagnant.

These data suggest that NK cells can be used clinically to treat solid tumors and may provide the greatest benefit when used in combination with surgery, radiotherapy, or chemotherapy by targeting the small population of CSCs that remain after these therapies.

<<5>>

EXPRESSION AND CHARACTERIZATION OF THE OTUBAIN-1 DEUBIQUITINATING ENZYME

Giselle Camarillo et al.

The ErbB family of mammalian receptor tyrosine kinases plays essential roles in propagating signals that regulate cellular proliferation, differentiation, motility, and survival. The aberrant overexpression of family member ErbB3 has been demonstrated to promote the onset and progression of breast cancer, and to contribute to the resistance of tumors to commonly employed therapeutics. These observations prompt questions concerning the mechanism by which breast tumor cells overexpress ErbB3, and whether anti-cancer therapeutic strategies may be developed that might suppress receptor overexpression. We have previously observed that the overexpression of Otubain-1 (Otb-1), a deubiquitinating enzyme, correlates with ErbB3 overexpression in breast cancer patient samples. Moreover, our observations suggest that the catalytic activity of Otb-1 may be required for receptor overexpression, raising the possibility that Otb-1-directed inhibitors could be employed to suppress ErbB3 overexpression in breast tumors. Here we have

expressed the Otb-1 protein in bacteria and have begun to develop a fluorescence resonance energy transfer (FRET)-based assay to assess its deubiquitinase activity. When fully developed, this assay will be employed in experiments aimed at screening and characterizing small molecule inhibitors of Otb-1 activity.

<<6>>
NEGATIVE REGULATION OF THE ERBB2 611-CTF BY LRIG1

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The epidermal growth factor family of receptor tyrosine kinases is composed of four members: EGFR, ErbB2, ErbB3, and ErbB4; these are membrane bound proteins that control important cell functions including cell cycle progression and differentiation through the activation of signaling cascades such as the Ras-mitogen-activated protein kinase (MAPK) and the phosphatidylinoditol 3-kinase (PI3K)-Akt pathways. Deregulated expression of the ErbB family by gene amplification and/or protein over-expression is associated with the development of a variety of cancer types. Notably, ErbB2 overexpression is observed in approximately 25% of human breast tumors. The negative regulator, LRIG1 is a transmembrane protein that can directly interact with all members of the ErbB family leading to the inhibition of the signaling pathways controlled by these receptors. In addition, LRIG1 *in vitro* experiments have shown that loss of LRIG1 is sufficient to drive an increase of ErbB2 protein levels and that the ectopic expression of LRIG1 decreases ErbB protein expression. Recently, a group of ErbB2 C-terminal fragments (CTFs) collectively known as p95HER2 have captured the attention of the scientific community not only because they are capable of inducing more aggressive tumors compared with those expressing full length ErbB2, but also because they have been implicated in therapeutic resistance. Among the p95HER fragments 611-CTF has the highest clinical significance because is hyperactive and possesses the capability to constitutively form homodimers and signal. In addition, 611-CTF has been associated with increased cell migration, tumor progression and metastasis. Currently, the mechanisms which lead to p95HER2 down-regulation are not well studied. The understanding of these mechanisms could ultimately lead to the development of new therapeutics which could improve the response rate of p95HER2-positive breast cancer. Here we show that LRIG1 can interact with 611-CTF, decrease receptor protein expression and reduce cell migration given by 611-CTF.

<<7>>
INCORPORATION OF BREAST CANCER RECEPTORS INTO NANOPARTICLES FOR DEVELOPMENT OF IMPROVED

Dennis Chang et al.

Cancer chemotherapy has curative potential in breast cancer, but intrinsic and acquired resistance is nearly universal, resulting in relapse. Over expression of growth factor receptors is common on the surface of breast cancer cells, and are the targets of several anticancer drugs. These receptors are not water-soluble and are difficult to study for the purpose of drug development. We are expressing this class of receptors, called ErbB proteins, and incorporating them into water-soluble nanoparticles that are amenable to biochemistry and drug screening studies. Synthesis and characterization of the ErbB-containing nanoparticles is in progress.

<<8>>
MICROSCOPIC ANALYSIS OF CELL DEATH BY METABOLIC STRESS-INDUCED AUTOPHAGY IN PROSTATE CANCER

Austin Changou, Holland Cheng, Richard Bold, Hsing-Jien Kung, and Frank Chuang

Autophagy promotes cellular survival against environmental stress and nutritional starvation. We have recently shown that some prostate cancers undergo metabolic stress and caspase-independent cell death following exposure to arginine deiminase (ADI, an enzyme that degrades arginine in tissue). The aims of our current investigation into the application of ADI as a novel therapy are to identify the components mediating tumor cell death, and to determine the role of autophagy (stimulated by ADI and/or rapamycin) on cell death. Using advanced fluorescence microscopy techniques including 3D deconvolution and structured-illumination superresolution imaging, we show that prostate tumor cells treated with ADI for

extended periods, die exhibiting a morphology that is distinct from caspase-dependent apoptosis; and that autophagosomes forming as a result of ADI stimulation contain DAPI-stained nuclear material. Fluorescence imaging (as well as cryo-electron microscopy) show a breakdown of both the inner and outer nuclear membranes at the interface between the cell nucleus and aggregated autophagolysosomes. Finally, the addition of N-acetyl cysteine (or NAC, a scavenger for reactive oxygen species) effectively abolishes the appearance of autophagolysosomes containing nuclear material. We hope to continue this research to understand the processes that govern the survival or death of these tumor cells, in order to develop methods to improve the efficacy of cancer pharmacotherapy.

<<9>>

IDENTIFICATION OF HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN K (HNRNP K) AS A BIOMARKER IN HEPATOCELLULAR CARCINOMA IN PATIENTS WITH CIRRHOSIS BY PROTEOMIC AND IMMUNOHISTOCHEMICAL STUDIES

Yantong Guo MD, PhD[1,2], Jingming Zhao MD[1], Jingtao Bi MD, Mingyi Chen MD, PhD[2].

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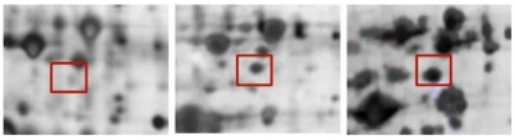
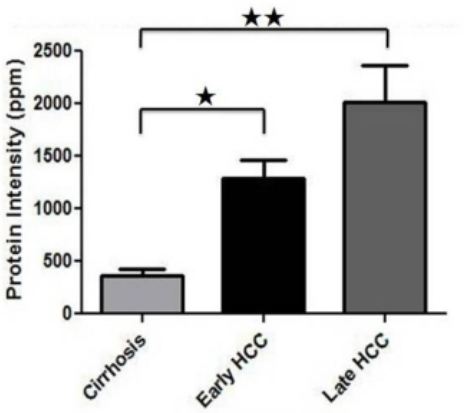
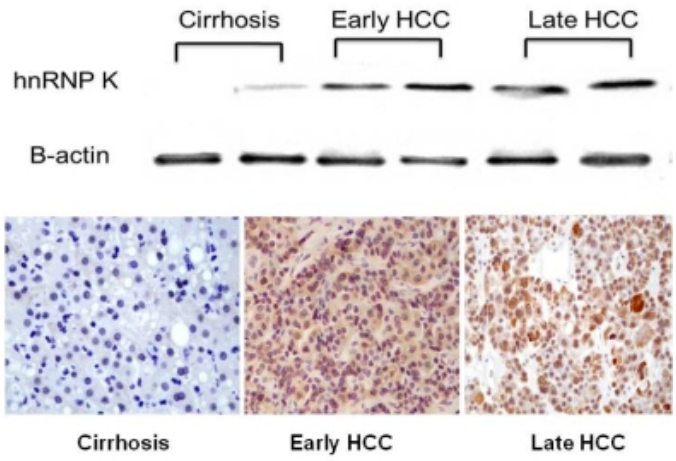
Background: Hepatocellular carcinoma (HCC), one of the most common malignant tumors worldwide, is particularly prevalent in Asian countries. Hepatitis B (HBV) and Hepatitis C (HCV)-associated cirrhosis are the major common risk factors of HCC. The diagnosis of HCC in a background of cirrhosis can be challenging. In this study, the biomarkers to distinguish HCC from cirrhotic nodules were studied.

Design: Frozen samples from 40 HBV-associated post-operative HCC patients and the clinicopathological data were collected. We used two-dimensional gel electrophoresis(2-DE) coupled with tandem mass spectrometry (MS) to study the proteins differentially expressed in HCC and the adjacent non-HCC cirrhotic tissue. Furthermore, the correlations between potential biomarkers and clinicopathological data of HCC were evaluated using bivariate correlation analysis.





Results: Heterogeneous nuclear ribonucleoprotein K (hnRNP K) was markedly upregulated in HCC compared to the non-HCC cirrhotic tissue. The overexpression of hnRNP K in HCC tissues was further confirmed by Western blot and immunohistochemistry. Receiver operating characteristic (ROC) curve analysis revealed that hnRNP K in combination with serum AFP was a sensitive (93.3%) and specific (96.0%) marker to detect HCC in HBV infected cirrhotic liver tissues.



Conclusion: The study revealed overexpression of hnRNP K in HCC which could be a potential diagnostic marker and a candidate target for therapy.

<<10>>

SELECTIVE T-TYPE CALCIUM CHANNEL BLOCKAGE FOR S-PHASE ENRICHMENT: A NOVEL CHRONOTHERAPEUTIC STRATEGY FOR GBM

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Cell cycle synchronization to enrich the chemo and radiosensitive cell cycle fraction of the tumor cell population is a potential therapeutic strategy which can be deployed in concert with standard chemoradiotherapy for brain tumors. Progression through the cell cycle is dependent on intracellular calcium signaling. Thus, modulating the amount of intracellular calcium available should provide a measure of control progression through the cell cycle. Calcium influx is governed in part by low voltage-activated T-type calcium channels. We hypothesize that *in vitro* use of the T-type calcium channel blocker mibefradil dihydrochloride will allow synchronization of C6 rat glioma cells.

Mibefradil maximum tolerance of 10.0 µM was determined by exposing C6 cells at 30-50% confluence to varying concentrations (0.1, 1.0, 10.0, 100.0 µM) in normal complete media (high glucose DMEM with 10% FBS and 1% penicillin-streptomycin) with dose-matched DMSO vehicle controls and assaying for cytotoxicity (lactate dehydrogenase release) and cell viability (WST-1 conversion to formazan). We used fluorescence-activated cell sorting (FACS) on propidium-iodide stained cells to quantify the effects of mibefradil on the cell cycle.

No significant differences were detected between DMSO vehicle controls and untreated cells receiving complete media alone. After 24h of treatment with 10.0 µM mibefradil, significant G0/G1 enrichment and S phase depletion indicated G1 arrest (compared to a known G1 arrest agent). Mibefradil treated cells maintained S phase enrichment up to 48h after mibefradil was washed out of the system, as compared to a decrease in S phase population over time in vehicle controls.

Future experiments will be performed to optimize the dosage and duration for maximum proliferative phase enrichment as well as quantification of calcium dependence.

<<11>>

CHROMATIN REMODELER RSF IN TELOMERE MAINTENANCE

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In addition to the specialized telomere-binding shelterin complexes, mammalian telomeres are bound by histones that assemble into nucleosomes. Telomeric histones contain repressive epigenetic marks characteristic of constitutive heterochromatin. The exact role of telomeric heterochromatin in telomere

maintenance, as well as how telomeric sequences determine the establishment of heterochromatin, is not well understood.

Our lab has identified a novel interaction between the shelterin protein Rap1 and Rsf-1, the histone binding subunit of the chromatin remodeling complex RSF (remodeling and spacing factor). In addition to Rsf-1, the RSF complex contains the ATPase SNF2H. In *Drosophila melanogaster*, dRsf-1 was reported to facilitate silent chromatin formation at pericentric heterochromatin by affecting H3K9me3 and H2Av levels. Using chromatin immunoprecipitation (ChIP) analysis, we were able to detect enrichment of SNF2h at telomeric sequences. In addition, we have demonstrated that Rsf-1 knockdown in human cells resulted in significant increase telomere recombination as measured by sister telomere exchange (T-SCE) frequency. We hypothesize that the RSF complex is recruited to telomeres and modulate the telomeric heterochromatin structures which repress telomere recombination.

<<12>>

UNDERSTANDING DYSKERATOSIS CONGENITA THROUGH THE GENERATION OF HUMAN CELL LINES HETEROZYGOUS FOR MUTANT TIN2

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The ends of linear eukaryotic chromosomes are composed of repetitive DNA sequences referred to as telomeres. In humans, the telomeric sequence is protected by the shelterin complex. TIN2, a component of the shelterin complex, is an essential gene with a role in protecting telomere integrity. Heterozygous mutations within the TIN2 gene have been identified in patients with Dyskeratosis Congenita (DC). DC is an inheritable disease that leads to bone marrow failure and is characterized by abnormally short telomeres. Mutations within the telomerase components, a multi-subunit reverse transcriptase that elongates telomeres, have also been found in different subtypes of patients with DC. These mutations prevent telomerase from functioning properly. It is currently unclear why mutations within TIN2 lead to extremely short telomeres in DC patients. I hypothesize that mutant TIN2 prevents telomerase from functioning properly at the telomeres. When disease-causing TIN2 mutants were overexpressed in telomerase-positive cell lines, the telomere length was comparable to wildtype TIN2. Therefore mutant TIN2 does not adversely affect wildtype TIN2. To study the TIN2 mutants at endogenous levels, human cell lines that are heterozygous for the TIN2 mutations need to be created. To facilitate homologous recombination between endogenous TIN2 and a mutant TIN2 gene-editing construct, I am utilizing zinc-finger nucleases. A successful pair of zinc-finger nucleases that cut exon 6 of TIN2 have been tested and confirmed. Transfection of the zinc-finger nucleases in addition to a TIN2 gene-editing construct yielded three verified mutant and six verified wildtype TIN2 clones. These cell lines ultimately will help determine the mechanism by which TIN2 mutations lead to the shortened telomeres in patients with Dyskeratosis Congenita and provide a successful treatment for these patients.

<<13>>

COLLECTING BLOOD FOR RESEARCH IN THE ASIAN AMERICAN COMMUNITY: A COLLABORATION BETWEEN THE UC DAVIS CANCER CENTER BIOREPOSITORY AND THE ASIAN AMERICAN NETWORK FOR CANCER AWARENESS, RESEARCH AND TRAINING (AANCART)

Regina Gandour-Edwards et al.

Background: The UC Davis Cancer Center Biorepository (CCB) and AANCART conducted three blood drives and tested blood from Asian Americans with limited English proficiency for Hepatitis B infection and diabetes to create a snapshot of these conditions in this community.

Methods: A specific AANCART consent form was developed, translated into Vietnamese, Hmong and Chinese and approved by the local IRB. Blood drives were organized as add-ons to events such as health fairs and special screenings. The UC Davis Department of Pathology and Laboratory Medicine provided phlebotomists and supplies. Interpreters consented participants with limited English proficiency. Participants received gift cards to local stores. Research participants were assigned study IDs and completed a one-page questionnaire in regards to age, gender, race, ethnicity and medical history such as diabetes or HEP B infection. Specimens were sent to the clinical lab for immediate testing or stored in the Biorepository -70C freezer for future research.

Results: We collected 585 specimens from 146 subjects. Fourteen of 146 tested postive for the Hepatitis B surface antigen. Five of 59 were considered diabetic based on the hemoglobin A1C test and thirteen showed high levels of glucose indicating an increased risk for diabetes.

Conclusions: Collaboration with AANCART to collect research specimens in the Asian American community aids in the goal to reduce cancer health disparities and promotes biospecimen collection for research. The support of the Clinical Laboratory and interpreters is critical for success.

<<14>>

DEVELOPMENT OF A UNIQUE HUMAN CANCER XENOGRAFT MODEL: ROLE OF THE UC DAVIS CANCER CENTER BIOREPOSITORY

Regina Gandour-Edwards, Neal Goodwin, Ryan Rodriguez, Irmgard Feldman, Pryia Singh, Richard Bold, Royce Calhoun, David Gandara, Ralph deVere White

BACKGROUND: The NSG mouse, developed by Jackson Laboratories (JAX), lacks mature B and T cells and functional NK cells making it a unique, immunodeficent model for human tumor xenografts. Since September, 2009, our biorepository (CCB) has provided 220 fresh human tumor samples for immediate engraftment utilizing our preoperative consenting protocol which ensures patient confidentiality by providing coded specimens to investigators.

METHODS: Informed consent is obtained by surgeons during a pre-operative visit and CCB is notified. On the day of surgey, surgical specimen is delivered fresh to pathology where the pathologist determines the availability of “remainder tissue” for research. Such tissue is placed into RPMI solution , coded by CCB and transferred to JAX . Tumor is engrafted within 1-2 hours into the NSG mice and monitored for tumor development. Tumors are harvested with 4-6 months at 1cm size with samples submitted for genomic analysis, histopathology and re-engraftment.

RESULTS: To date, sixty patient derived xenograft (PDX) models have been successfully established of diverse tumor types including 20 lung cancer, 10 glioblastoma and 10 pancreatic cancer tumors. Morphologic comparison of the patient and mouse tumors has demonstrated remarkable fidelity. Genomic analysis for EGFR and KRAS mutations in has shown excellent correlation in 10 lung samples to date.

CONCLUSIONS: Following OHRP guidelines for informed consent and patient confidentiality, the biorepository has become an effective resource for providing fresh human tumor tissue to generate a PDX bank of diverse tumor types for basic research.

<<15>>

COMBINING SELECTIVE ACETYLATION AND GLYCOSYL IODIDE CYCLIC ETHER GLYCOSYLATION TO ACHIEVE BRIEF SYNTHESES OF FUNCTIONALIZED GB-3 TRISACCHARIDES

*Hsiao-Wu Hsieh and Jacquelyn Gervay-Hague**

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Oligosaccharides are known to be important in cell-to-cell communication processes that initiate many biological processes. Isolating biologically relevant carbohydrates from biological sources is challenging - as is the chemical synthesis, which inevitably requires multiple steps and protecting group manipulations. A methodology of selective acetylation has been developed to shorten the synthesis of complex oligosaccharides. The reaction uses common chemical reagents under microwave assistance to generate partially acetylated building blocks, which otherwise would take many steps to synthesize via conventional methods. Glycosyl iodides exhibit good reactivity and selectivity during the glycosylation reactions. When incorporated with the reaction of introducing cyclic ethers to the anomeric position, functionalized oligosaccharides can be prepared in a relatively short synthetic route and reasonable yields.

<<16>>

EVALUATION OF SELECTIVE INHIBITORS OF NUCLEAR EXPORT (SINE) CRM1 INHIBITORS FOR THE TREATMENT OF RENAL CELL CARCINOMA (RCC)

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Background: For the ~30% of patients who present with metastatic RCC, multi-kinase inhibitors have been used with only moderate success: progression-free survival remains at only up to two years. Thus it is imperative to discover novel therapeutic approaches for metastatic RCC. We asked whether (1) inhibitors of nuclear exporter attenuate key cell cycle regulatory and apoptotic molecules and (2) whether these inhibitors exert salutary effects in a human RCC xenograft mouse model.

Methods: Four RCC cell lines with distinct genotypes, and primary normal human kidney (NHK) cell lines, were used. The cells were treated with the chromosome region maintenance protein 1 (CRM1) inhibitors KPT-185 or 251 and MTT assays were performed. In addition, cell cycle analyses, immunofluorescence for p53 and p21, and immunoblotting for CRM1, p53, p21, p27, and p-MDM2 were performed. Caki-1 xenograft mice were treated with vehicle, the orally-available CRM1 inhibitor KPT-251, or sorafenib to analyze tumor volume and on-target effects.

Results: KPT-185 and -251 reduced CRM1 protein levels in RCC cells. KPT-185 caused greater cytotoxicity in RCC cells but less toxicity in NHK cells than sorafenib, suggesting a possible clinical advantage of KPT-185 over sorafenib. By FACS analysis, we showed that KPT-185 arrests the cell cycle in both G2/M and G1, and increased the sub-G0 cell population. KPT-185 and 251 both increased nuclear levels of the TSPs p53 and p21 in RCC cells. *In vivo*, KPT-251 inhibited Caki-1 xenografts in mice compared to both vehicle and sorafenib without obvious systemic adverse effects.

Conclusions: We introduce a novel therapeutic approach to the RCC treatment based on the nuclear export inhibition of TSPs and key cell cycle regulatory proteins. CRM1 inhibition leads to forced nuclear retention, and thereby activation, of several p53-pathway proteins, leading to cell cycle arrest and apoptosis in RCC growth inhibition *in vitro* and *in vivo*.

<<17>>

FUNCTIONAL P53 DETERMINES DOCETAXEL SENSITIVITY IN PROSTATE CANCER CELLS

Chengfei Liu[1], Yezi Zhu[1,2], Wei Lou[1], Nagalakshmi Nadiminty[1], Xinbin Chen[2], Xubao Shi[1], Ralph W. deVere White[1], and Allen C. Gao[1,2]¶

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Objectives: Docetaxel is the first line treatment for castration resistant prostate cancer (CRPC). However, docetaxel resistance rapidly develops. Identifying the critical mechanisms giving rise to docetaxel resistance is the major challenge in advanced prostate cancer.

Methods: The effects of docetaxel on human DU145, PC3, LNCaP and C4-2 prostate cancer cells were examined in cell culture, and p53 expression were analyzed by Western blot analysis. The potential role of p53 in docetaxel sensitivity in prostate cancer cells was tested by either p53 silencing using shRNA or p53 overexpression by introducing wild-type p53.

Results obtained: We found that DU145 (mutant p53) and PC3 (p53 null) cells were less sensitive than LNCaP and C4-2 cells expressing functional p53 in response to docetaxel. Docetaxel treatment induces considerably higher apoptosis in LNCaP and C4-2 cells than in DU145 and PC3 cells in a dose dependent manner. Docetaxel increases the levels of ser15 phosphorylation of p53 in a dose dependent manner in both LNCaP and C4-2 cells, while has no effect on the levels of ser15 phosphorylation of p53 in DU145 cells. These results suggest that p53 phosphorylation is associated with docetaxel sensitivity in prostate cancer cells. To further confirm whether p53 activation can induce cell sensitivity to docetaxel treatment, we used p53 shRNA to knock down p53 expression in C4-2 cells and determined the cells response to

docetaxel treatment. Knockdown of p53 significantly down regulated p53 phosphorylation and blocked docetaxel induced apoptotic cell death compared to the vector control. To further confirm this observation, we established a stable knock out p53 in C4-2 cells. Down regulation of p53 in the stable p53 knock out C4-2 cells significantly inhibited docetaxel induced apoptotic cell death. We also used wild-type (WT) p53 to over express p53 in DU145 cells, and found that expression of WT-p53 in DU145 cells increased their sensitivity to docetaxel.

Conclusions: These results demonstrate that docetaxel induces p53 phosphorylation and that p53 status is a crucial determinant of docetaxel sensitivity in prostate cancer cells**.**

Funding sources: This work was supported in part by grants from NIH CA118887, DOD PC080538, and VA Merit Award I01 BX000526.

<<18>>

ANDROGEN RESPONSIVE MIR-148A ALTERS CELL CYCLE REGULATION BY TARGETING P27 IN PROSTATE CANCER CELL LINES

Alan P. Lombard[1,2], Stephen J. Libertini[2,3], Alyssa C. Thunen[2], Keeley B. Fornaci[2], Maria Mudryj[2,3]

[1] BMCDB Graduate Group, University of California, Davis; [2] Department of Medical Microbiology and Immunology, University of California, Davis; [3] Veterans Affairs-Northern California Health Care System, Mather, California 95655

Prostate cancer is a commonly diagnosed malignancy and represents the second leading cause of cancer-related death in US men. Prostate cancer is initially dependent on androgens for survival but often progresses to an androgen independent state for which few treatments exist. Androgen signaling is mediated through the androgen receptor (AR) but the downstream mechanisms that allow the androgen receptor to influence cellular proliferation remain to be fully understood. Further research elucidating these pathways is needed to discover novel therapeutic options for this disease. It has been shown that the AR represses the function of the cyclin dependent kinase inhibitor p27 but the way in which this happens is not completely known and seems to be multi-pronged. Research from Murata et al. demonstrated that miR-148a is an androgen responsive microRNA. Furthermore, it was shown by Guo et al. that miR-148a targets p27 in gastric cancer. Here we attempt to link these lines of research and uncover a novel pathway in which the relationship between the AR and p27 is mediated by miR-148a. We validated previous results showing that inhibition of androgen signaling leads to an increase in p27 expression at both the protein and mRNA levels in two separate androgen responsive prostate cancer cell lines, LNCaP and 22Rv1. Treating cells with 10nM DHT leads to increased levels of miR-148a and attenuating androgen signaling leads to decreased levels of miR-148a as expected. Using a microRNA mimic, we demonstrate the ability of miR-148a to target p27 expression at both the protein and mRNA levels and further, that it can rescue the attenuated androgen signaling phenotype and increase cyclin-A levels suggesting positive regulation of the cell cycle. These results indicate that miR-148a plays a role in linking the AR to the cell cycle by repressing p27.

<<19>>

STABILITY OF MIRNA IN HUMAN URINE SUPPORTS THEIR BIOMARKER POTENTIAL IN UROLOGIC CANCERS

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Serum microRNAs (miRNAs) are showing utility as biomarkers in a variety of malignancies, but while these molecules have been cursorily examined in urine, a rigorous evaluation of their stability and thus their utility in this harsh biofluid is lacking. Previous work in our laboratory has focused on assessing urinary metabolite biomarkers of RCC, and in the current study we evaluate the stability of miRNAs in urine under clinically-relevant collection and storage procedures. Four healthy individuals of each gender provided clean catch urine samples which were stored at room temperature or at 4^{o}C for up to 5 days, or were subjected to up to 10 freeze-thaw cycles at -80^{o}C to room temperature. Levels of two representative urinary miRNAs, miR-16 and miR-21, were evaluated by qRT-PCR under each condition. We now show that,

under all 3 conditions, there was a surprising degree of stability of miRNAs in the urine: over the 5-day period of storage at RT, there was 35% of the initial amount remaining; at 4^{o}C, there was 42-56% remaining; and by the end of the 10 freeze-thaw cycles, the amount remaining was 23-37%. The average levels of miRNA by the end of the storage periods were well within the readily detectable C_T value of 35, and both miRNAs examined, which showed no sequence homology, showed degradation at approximately the same rate. Furthermore, when the urine was treated with trypsin, there was no change in miRNA levels suggesting that miRNAs do not require association with exosome-associated proteins for their urinary stability. This study shows that miRNAs are relatively stable in the harsh urinary environment under a variety of storage conditions and thus are highly amenable and should be further pursued as robust clinical markers of malignancy.

<<20>>

LRIG3 PROMOTES WNT SIGNALING IN BREAST CANCER CELLS THROUGH STABILIZATION OF THE WNT CO-RECEPTOR, LRP6

Frank Mercado, Michael Astudillo and Colleen Sweeney

Department of Biochemistry & Molecular Medicine, UC Davis School of Medicine

The Wnt ligands are family of secreted glycoproteins involved in a variety of cellular processes including proliferation, survival, cell fate and polarity. Although best studied in embryonic development, Wnts also have important functions in adults, and altered Wnt signaling has been linked to several types of cancers. In mammary tissues, Wnt signaling plays an important role in development of the mammary gland as well as in stem cell renewal. Wnt signaling can operate through a variety of pathways, the best understood of which is the canonical/B-catenin pathway. Enhanced beta catenin staining (~60%) has been found in human breast cancer specimens, and evidence indicates that when the Wnt pathway is aberrantly activated it leads to mammary carcinogenesis. In the absence of Wnt ligand, beta catenin is degraded; however in the presence of Wnt, a bridge is formed between the ligand, Frizzled (Fz) a seven transmembrane receptor, and low density lipoprotein (LRP)5 or LRP6 co-receptors. Work in our lab focuses on the Leucine rich repeat and immunoglobulin like domain (LRIG) family of proteins 1-3. LRIG1 has been characterized as a tumor suppressor and works by destabilizing a variety of receptor tyrosine kinases, including ErbB and Met, while the actions of the other LRIGs have not been clearly identified. Here we show that ectopic expression of the third member of the LRIG family, LRIG3, can increase canonical Wnt signaling, while silencing of LRIG3 causes a decrease in beta catenin levels. We also show that LRIG3 may be affecting Wnt signaling by specifically stabilizing the LRP6 receptor. Recently, LRP6 has been shown to be upregulated in a sub-population of human breast cancers, and silencing of LRP6 shows a marked inhibition of Wnt signaling. Increased LRP6 expression is sufficient to trigger Wnt signaling, and stabilization of the receptor by LRIG3 may play a role in the development in this sub-population of breast cancers.

<<21>>

SOCIAL AND CULTURAL INFLUENCES ON TOBACCO-RELATED HEALTH DISPARITIES AMONG SOUTH ASIANS IN THE UNITED STATES

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Background: South Asians are the 2nd largest Asian subgroup & fastest growing minority population in the U.S. Population-based research in the U.S. has concluded that tobacco does not contribute to existing disparities; these studies did not inquire about indigenous products commonly used by this group. Local surveys including culturally-specific tobacco demonstrate higher use rates, suggesting more consistent associations with tobacco-related disparities.

Methods: Focus groups (n=100) were conducted in 3 South Asian ethnic enclaves in the U.S. Participants were separated by key demographic variables but included diversity of religion and national origin. Deductive methods were used in content analyses to qualitatively describe patterns & predictors of tobacco use.

Results: A large number of culturally-specific tobacco products are commonly used by South Asians. Knowledge of product-specific risks was lacking or inaccurate. Culturally-specific products were differentially considered to have beneficial attributes. Use of South Asian products was ascribed social & cultural value, seemingly superseding perception of impacts on health. South Asians use these products to preserve and express ethnic identity in a new dominant culture, and to distinguish themselves from mainstream society and other minority groups. Product use is often a symbolic behavior to maintain tradition, engage in cultural celebration, serve as reminders of common heritage, and facilitate socialization among persons with a shared ethnic identity.

Conclusions: Many cultural factors govern tobacco use among South Asians in the United States and are not included in typical risk factor surveillance instruments. Measuring the prevalence and correlates of non-traditional tobacco use is pivotal to understanding the true contribution of all forms of tobacco to health disparities, and will facilitate identification of targets for intervention. For understudied minority groups, the role of social identity may strongly influence at-risk behaviors. Broader implications include extrapolation of findings to other culturally-framed behaviors among groups underrepresented in health disparities research.

<<22>>

MOVING TOWARDS A TRUE DEPICTION OF TOBACCO USE AMONG SOUTH ASIANS: ANALYSES FROM THE CALIFORNIA ASIAN INDIAN TOBACCO USE SURVEY

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[1]Center for Tobacco Control Research & Education, University of California, San Francisco; [2]California Tobacco Control Program, California Department of Public Health,; [3]Division of General Internal Medicine, Department of Medicine, University of California, Davis

Objective: Despite disproportionate burdens of tobacco-related disease, past reporting has largely concluded that tobacco use does not contribute to excess burden of illness among Asian Indians in the United States. Besides cigarettes, Asian Indians use culturally-specific tobacco products. We examine Asian Indian tobacco behavior in California, which has had a long-standing tobacco control program and the largest Asian American population.

Methods: We used 2004 California Asian Indian Tobacco Use Survey (CAITUS) statewide data (N=1,782) to determine the prevalence of culturally-specific tobacco product use. Be creating a composite variable comprised of four culturally-specific tobacco products: bidis, hookah, paan, and gutka, we used multivariate regression for association with socioeconomic status, acculturation and religious affiliation.

Results: Culturally-specific tobacco use among Asian Indians was higher than that of cigarette smoking (13.4% vs. 5.5%). Disparities in current use rates between men and women were narrower than observed for cigarette smoking. Asian Indian men, those with higher incomes and education, immigrants, those who spoke an Indian language at home, and non-Sikhs were more likely to be users of culturally-specific smokeless tobacco.

Conclusions: These analyses confirm that use of tobacco, particularly culturally-specific products, is a significant problem among Asian Indians in California, paralleling patterns found in other Asian Indian ethnic enclaves domestically and globally. Currently, statewide efforts do not focus on decreasing the prevalence of culturally-specific tobacco products. Enhanced surveillance measures and targeted interventions, especially for culturally-specific smokeless products, are needed to address tobacco-related disparities among Asian Indians in California and nationwide.

<<23>>

COMPUTATIONAL MODELING AND ANALYSIS OF NEGATIVE FEEDBACK AND COUPLED SIGNALING IN THE SMAD-DEPENDENT TGF-B SIGNAL TRANSDUCTION PATHWAY

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The transforming growth factor β (TGF-β) and bone morphogenetic protein (BMP) ligands, members of the TGF-β superfamily, control multiple cellular processes central in regulating embryonic development and

tissue homeostasis, including differentiation, proliferation, motility, and apoptosis (Massagué, 2008). Mutations affecting the molecular components along the TGF-β signaling pathway are associated with a variety of cancer types (Levy & Hill, 2006). Although an understanding of the pathway could seem apparently straightforward, its complexity greatly escalates due to a high degree of receptor promiscuity and the coupling of intracellular signaling mediators (Feng & Derynck, 2005). Computational modeling allows us to control multiple steps throughout the signaling cascade and elucidate the effects of alterations of the pathway components in the behavior of the system.

We have developed a comprehensive, mechanistic mathematical model of the TGF-β signaling pathway, focusing on the role of an inhibitory-Smad-based negative feedback loop in regulating the system response through two parallel intracellular signaling channels (Nicklas & Saiz, 2012a). We show that this effect is sufficient to explain the distinct dynamics observed in human keratinocytes (HaCaT), bovine aortic endothelial cells (BAECs), and mouse myoblasts (C2C12). In addition, we examine the impact of crosstalk between the channels upon induction by TGF-β and BMP. Using a global sensitivity analysis, we have demonstrated that variations in the negative feedback loop differentially affect the sensitivity of several processes to mutations of the pathway components (Nicklas & Saiz, 2012b). Altogether, our analysis provides new insights into variability within the TGF-β signal transduction network, further elucidating the underlying mechanisms responsible for its normal and pathological behavior.

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- Levy L, Hill CS (2006) Alterations in components of the TGF-β superfamily signaling pathways in human cancer. Cytokine Growth Factor Rev 17: 41-58
- Massagué J (2008) TGFβ in Cancer. Cell 134: 215-230
- Nicklas D, Saiz L (2012a) Computational modeling of Smad-mediated negative feedback and crosstalk in the TGF-β superfamily network. Submitted
- Nicklas D, Saiz L (2012b) Effect of negative feedback architecture on dynamics, robustness, and sensitivity in the TGF-β signal transduction pathway. Preprint

<<24>>

IDENTIFICATION OF NRDP1 AS A NOVEL ANDROGEN RECEPTOR TRANSCRIPTION TARGET DIFFERENTIALLY REGULATED IN ANDROGEN-DEPENDENT AND INDEPENDENT PROSTATE CANCER

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Background: The ErbB receptor tyrosine kinase family regulates proliferation and survival in prostate cancer (PCa). We recently showed that ErbB3 plays a significant role in increasing androgen receptor (AR) transcriptional activity and in causing castration resistant PCa (CRPC). In addition, AR maintained castration sensitivity by suppressing ErbB3 levels through transcriptional regulation of E3 ubiquitin ligase Nrdp1, while loss of AR regulation of Nrdp1 resulted in an unrestricted surge in ErbB3 levels, and cell growth.

Results: The promoter region of *Nrdp1* contains three androgen response elements (ARE) - one located 215 aa upstream (ARE.03), and two within an internal promoter (ARE.01 and ARE.02). CHIP revealed AR binding in PCa cells to ARE.03 and ARE.02. AR binding to ARE.03 was found to be androgen regulated in androgen-dependent LNCaP PCa cells, AR binding in the presence of androgens. Luciferase assay to determine AR transcriptional activity on ARE.03 showed significant response to androgens, whereas mutation abolished AR transcriptional activity. CHIP also showed that the AR failed to bind to ARE.03 in C4-2 and LNCaP-AI, CRPC sublines of LNCaP cells, although no mutations in these regions were identified. Luciferase assay in LNCaP-AI showed decreased AR transcriptional activity on ARE.03 compared to LNCaP. We earlier showed that cleavage of the full-length FlnA to a 90 kDa fragment (FlnA16-24) and its nuclear localization, maintained androgen dependence. Transfection of FlnA16-24 in

C4-2 cells restored AR binding to ARE.03, indicating that this AR binding protein is required for AR-mediated transcription of Nrdp1.

Conclusions: We identified *Nrdp1* as a novel target of AR transcriptional activity in androgen-dependent but not in CRPC cells. Our data indicate that the AR-binding protein FlnA16-24 is required for AR binding to the *Nrdp1* promoter and that loss of this protein results in the failure of AR to regulate *Nrdp1* transcription, resulting in unrestricted increase in ErbB3.

<<25>>

IMMUNOSTIMULATORY CANCER IMMUNOTHERAPY REGIMENS INDUCE SUBSEQUENT POTENT IMMUNOSUPPRESSIVE RESPONSES

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We have previously shown that strong immune stimulation using agonistic αCD40 and IL-2 as well as other immunotherapy (IT) regimens result in the induction of robust $CD8^+$ T cell-mediated antitumor responses that are capable of inducing complete tumor regression in various advanced tumor models. We also observed a marked increase in peripheral regulatory T cells with a concordant increase in activation induced cell death of conventional $CD4^+$ T cells during and after therapy. Given this milieu activating and inhibitory signals, we sought to determine the ability of T cells to react to various stimuli during strong immune stimulation such as IT used in cancer treatment. Splenocytes from IT treated mice exhibited significantly blunted proliferative responses to TCR engagement but not cytokine stimulation. CFSE analysis revealed that while CD8+ T cell proliferation and activation marker upregulation were comparable to controls, CD4 T cells failed to proliferate and upregulate CD25. We next investigated primary CD4 responses by mixed lymphocyte reactions (MLR). Mice receiving IT lost the ability to proliferate in primary MLRs compared with controls indicating a profound state of antigen-unresponsiveness. Loss of MLR occurred early during the course of immune stimulation and regardless of combination of αCD40/IL-2 or either treatment singly suggesting that the naïve CD4+ T cell paralysis was a result of strong stimulation. Further analysis of the naïve CD4+ T cell population revealed a concomitant upregulation of SOCS3 in the T cells following IT. SOCS3 is a negative regulator of JAK/STAT signaling, including STAT5 which contributes to CD25 upregulation following TCR mediated activation. Consistent with this, STAT5 phosphorylation was diminished in CD4 T cells restimulated following IT further suggesting a role for SOCS3 in the naïve CD4 paralysis. These data demonstrate that immunostimulatory regimens used in cancer treatment, while inducing potent initial anti-tumor effects, also result in subsequent immunosuppression and immune paralysis affecting primary immune responses.

<<26>>

IN VIVO EVALUATION OF THE GROWTH INHIBITORY FUNCTION OF LRIG1 IN THE MAMMARY GLAND

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Lrig1 is a transmembrane leucine-rich repeat protein and a negative regulator of oncogenic receptor tyrosine kinases such as the ErbB family members. The Sweeney lab recently demonstrated that under-expression of Lrig1 predicts poor prognosis in breast cancer. Breast cancer patients with high Lrig1 expression show significantly longer relapse-free survival, identifying Lrig1 as a new prognostic marker. Recently, Lrig1 was found to be a tumor suppressor in intestinal tissue. It was shown that Lrig1 null mice develop ErbB receptor-dependent duodenal adenomas. Based on Lrig1's function as a negative regulator of RTKs and its reported tumor suppressor activity in intestinal epithelium, we hypothesized that Lrig1 knockout mice would be susceptible to mammary tumorigenesis. To characterize the function of Lrig1 in the mammary gland, we compared mammary development in Lrig1 wild type (+/+) and null (-/-) mice at regular intervals from 4 to 12 weeks. Mice were screened for evidence of hyperplasia, pre-malignant lesions or tumors by whole mount analysis across age and the intensity of specific proteins was surveyed

by western blot. Although these studies are ongoing, early results indicate an increase in the amount of Lrig1-target proteins by western blot in the Lrig1-/- mice as opposed to the Lrig1+/+ mice mammary gland tissue. In addition, a number of proliferative lesions were encountered in the mammary gland whole mounts of the Lrig1 -/- mice, but not that of the Lrig1+/+ mice, which strongly suggests that Lrig1 plays a tumor suppressor role in the mammary gland.

<<27>>

ROLE OF H2-RELAXIN IN INDUCING RADIATION RESISTANCE THROUGH P53 PATHWAY

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Prostate cancer is the most common form of cancer in men over 50 and about half of these patients choose radiation therapy as their initial form of treatment. However, resistance to radiation therapy is observed in ~40% of men receiving this therapy. About half of all human malignancies, including prostate cancer, express p53 mutations, and numerous reports indicate p53 mutation as a major molecular cause of radiation resistance. Previous studies indicated R273H as a hot spot mutation in prostate cancer, and the goal of these studies was to investigate downstream targets of p53 affected by this mutation that can be manipulated to overcome radiation resistance. LNCaP cells expressing wild type p53 or the R273H mutation (LNCaP-R273H) were treated with increasing doses of radiation, and the LNCaP-R273H cells were significantly more resistant to radiation as determined by clonogenic assay. Microarray analysis of these cells showed that in LNCaP cells, radiation therapy induced disruption of genes that regulate cytoskeletal structure, whereas the same genes were unaffected in LNCaP-R273H cells. Previous studies showed that the insulin-like peptide hormone H2-Relaxin (RLN2) is a downstream effector of p53R273H, hence we investigated whether resistance to radiation-induced disruption of the actin cytoskeleton was mediated by RLN2. Comparison of the effects of R273H vs RLN2 showed significant overlap. In particular, we observed differential expression of several genes belonging to the Wnt family of signaling peptides and their conjugate transmembrane receptors, the Frizzleds (Fzds). In addition, several stem cell markers were also upregulated in the RLN2/R273H expressing cells, supporting previous reports of resistance to radiation caused by an increase in the stem cell population. These data indicate correlation between cytoskeletal stability, stem cell regeneration and radiation resistance; hence, it is of interest to determine whether treatments that disrupt the actin cytoskeleton can induce sensitivity to radiation in prostate cancer cells.

<<28>>

PROSTATE EPITHELIUM-SPECIFIC R270H MUTATION IN THE P53 GENE INDUCES PROSTATIC INTRAEPITHELIAL NEOPLASIA IN MICE

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Tp53 mutations are common in prostate cancer (CaP), occurring with a frequency of ~30% and ~70% in localized and metastatic disease respectively. In vitro studies have determined several common mutations of *Tp53* that have specific gain of function properties in addition to loss of function, including the ability to promote castrate resistant growth of CaP cells in some contexts. To date, a lack of suitable mouse models has prohibited investigation of the role played by *p53* mutations in mediating CaP progression in vivo. Here we describe the effects of conditional expression of a mutant *p53* that is equivalent to the human hotspot R273H into the prostate epithelium of mice. Heterozygous "*p53*$^{LSL\cdot R270H/+}$" (129S4(Trp53^{tm3Tyj})*;Nkx3.1cre"* (129S(Nkx3-1^{tm3CreMms}) mice with prostate–specific expression of the *p53.R270H* mutation (*p53*$^{R270H/+}$ *Nkx3.1cre* mice) bred on to a FVB/N background via speed congenesis to produce strain FVB.129S4(Trp53$^{tm3Tyj/wt}$);FVB.129S(Nkx3-1$^{tm3CreMms/wt}$) and littermate genotype negative control mice.

These mice had significantly increased incidences of prostatic intraepithelial neoplasia (PIN) lesions that appeared earlier compared to the Nkx3.1 haploinsufficient (*Nkx3.1cre het)* littermate mice that did not express the *Tp53* mutation. PIN lesions in these mice showed consistent progression, and invasive adenocarcinoma that evolved into a high grade, sarcomatoid or epithelial-mesenchymal transition (EMT) phenotype. PIN lesions were similar to those seen in PTEN conditional knockout mice, with evidence of AKT activation concomitant with neoplastic proliferation. Meanwhile, the invasive tumor phenotype was unlike any previously described mouse model of prostatic neoplasia. These data indicate the $p53^{R270H}$ mutation plays a role in CaP initiation. This finding has not previously been reported. Further characterization of this model, particularly in a setting of androgen deprivation, should allow further insights into the mechanisms by which the $p53^{R270H}$ mutation mediates CaP progression.

<<29>>

COLLECTIVE PROSTATE CANCER CELL INVASION DEPENDS ON N-CADHERIN

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Cancer cell invasion is the critical first step of metastasis, yet, little is known about how cancer cells invade and initiate metastasis in a complex extracellular matrix. Using a cell line from bone metastasis of prostate cancer (PC3), we analyzed how prostate cancer cells migrate in a physiologically relevant 3D Matrigel. We found that PC3 cells migrated more efficiently as multi-cellular clusters than isolated single cells, suggesting that the presence of cell-cell adhesion improves 3D cell migration. Perturbation of N-cadherin function by transfection of either a dominant negative construct or shRNA specific to N-cadherin abolished collective cell migration. Interestingly, PC3 cells do not express α-catenin, an actin binding protein in the cadherin complex. When the full-length α-catenin was re-introduced, the phenotype of PC3 cells reverted back to a more epithelial phenotype with a decreased cell migration rate in 3D Matrigel. Interestingly, we found that the N-terminal half of α-catenin was sufficient to suppress invasive phenotype. Taken together, these data suggest that the formation of N-cadherin junctions promotes 3D cell migration of prostate cancer cells, and this is partly due to an aberrant regulation of the N-cadherin complex in the absence of α-catenin.

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UCDAVIS COMPREHENSIVE CANCER CENTER

CANCER BIOLOGY RESEARCH SEMINARS

Thursdays, 9 a.m. to 10 a.m.

Coordinators: Dr. Hsing-Jien Kung, Dr. Hongwu Chen, Dr. Tianhong (Tina) Li, Dr. Paramita Ghosh, Dr. Chong-Xian Pan, Dr. Colleen Sweeney,

DATE	SPEAKER	TOPIC	LOCATION
Sept. 13, 2012	**Dr. Matthew Ellis** Washington University *Host: Dr. Tina Li*	*Genomic Landscape of Breast Cancer*	UCDMC, Cancer Center Auditorium
Sept. 20	**Dr. Richard Levenson** UC Davis *Host: Dr. Tina Li*	*Path, Present, and Future*	UCDMC, Cancer Center Auditorium
Sept. 27 & 28	***Annual Cancer Research Symposium***	*Various speakers* *Registration: http:/ucdresearchsymposium.eventbrite.com*	UCDMC, Cancer Center Auditorium & Courtyard Marriott
Oct. 4	**Dr. XiuWei Yang** U Kentucky *Host: Dr. Colleen Sweeney*	*Tetraspanin CD151 Contributes To Breast Cancer Malignancy By Facilitating The Cross-Talk Between Integrins and ErbB Receptors*	UCDMC, Cancer Center Auditorium
Oct. 11	**Dr. Michael Mingzhao Xing** Johns Hopkins U SOM *Host: Dr. Hongwu Chen*	*Genetic Alterations in Thyroid Cancer: Clinical Implications*	UCDMC, Cancer Center Auditorium
Oct. 18	**Dr. Lin He** UC Berkeley *Host: Dr. Wolf Heyer*	*miRNAs at the crossroads between cancer and pluripotency*	UCDMC, Cancer Center Auditorium
Oct. 25	**Dr. Lilly Bourguignon** UC San Francisco *Host: Dr. Hongwu Chen*	*Hyaluronan-CD44 Interaction with Nanog/Oct4/Sox2 Signaling Promotes MicroRNA-302 Function and Chemo-therapy Resistance in Cancer Stem Cells*	UCDMC, Cancer Center Auditorium
Nov. 1	**Dr. Jin Zhang** UC Davis Vet. Med. *Host: Dr. Xinbin Chen*	*Probing the Role of the P53-RNPC Loop in Tumor Suppression and Longevity*	UCDMC, Cancer Center Auditorium
Nov. 8	**Dr.** to be filled *Host:*	*TBA*	UCDMC, Cancer Center Auditorium
Nov. 15	**Dr. Kathleen O'Connor** U Kentucky *Host: Dr. Colleen Sweeney*	*Modulating Rho GTPase Function during Carcinoma Invasion: Tales from an Integrin*	UCDMC, Cancer Center Auditorium
Nov. 22/23	**Thanks giving holidays**	=====	=====
Nov. 29	**Dr. Esther Chang** Georgetown Univ. *Host: Dr. Chong-Xian Pan*	*Is Dual Targeting Critical for the Design of An Effective Cancer Therapy?*	UCDMC, Cancer Center Auditorium
Dec. 6	**Dr. Frank Slack** Yale *Host: Dr. Paramita Ghosh*	Topic: *MicroRNAs*	UCDMC, Cancer Center Auditorium
Dec. 13	**Dr. Carol A. Lange** U Minnesota *Host: Dr. Hongwu Chen*	*Topic: Breast Cancer*	UCDMC, Cancer Center Auditorium

Refreshments will be provided