Speaker Abstracts

Spatial-Domain Low-Coherence Quantitative Phase Microscopy for Cancer Detection

Yang Liu, Pin Wang, Rajan Bista, David Y. Lo, Kevin Staton, Wei Qiu, Rohit Bhargava, Walid Khalbuss, Kevin Mcgrath, Kenneth E. Fasanella, Lin Zhang, Randall E. Brand

University of Pittsburgh, Pittsburgh, Pennsylvania

Cancer is typically diagnosed based on the microscopic examination of morphological changes in the cell and tissue, particularly alterations in nuclear structure. Despite the well-established nuclear morphological features in cancer cells, subtle changes in the nuclear architecture may not be easily detectable with conventional microscopy, especially when only a small amount of human cell or tissue samples are available for examination or in the early course of the tumor development, leading to potential delay of definitive diagnosis or repeating procedures to obtain additional samples. To overcome these challenges, techniques with increased sensitivity that can detect subtle pathologically undetectable cellular alterations with significant clinical applicability are urgently needed.

With the funding support of the R21 from IMAT program, our group has developed a novel optical technique Spatial-domain Low-coherence Quantitative Phase Microscopy (SL-QPM) that has overcome the technical difficulties by adapting a low-coherence thermal light source and common light path configuration that effectively remove the notorious noise associated with quantitative phase microscopy. This instrument produces a speckle-free two-dimensional image that quantifies the spatial variation of optical path length differences or refractive index of the sub-cellular architecture with nanoscale sensitivity (~1 nm).

To demonstrate the capability of SL-QPM in detecting subtle, sub-cellular structural alterations that are invisible to conventional pathology, we performed a proof-of-concept experiment with pathologically normal intestinal epithelial cells from an animal model of intestinal carcinogenesis APC/Min mouse model. We analyzed the cell nuclear architectural characteristics and refractive index properties in the intestinal epithelial cells prepared with standard clinical cytology and histology protocols. We show that despite the indistinct and normal-appearing pathological features in intestinal epithelial cells from the 4 month-old APC/Min mice with tumors and the wild-type mice, SL-QPM can detect the significant changes in nanoscale nuclear architectural characteristics quantified by the optical path length and refractive index.

To confirm the significance of nanoscale nuclear architectural characteristics and refractive index and evaluate their applicability in clinical cancer diagnosis, we conducted pilot studies in three tumor types: pancreatic, esophageal and breast cancers. We studied cytology specimens in 40 patients with suspicious pancreatic solid lesions undergoing endoscopic ultrasound guided fine-needle aspiration, and 30 patients with Barrett's esophagus or dysplasia and cancer with cytology brushing of cardia mucosa at gastroesophageal junction. We further evaluated breast histology specimens from 70 women undergoing surgical procedures. We found that the quantification of nanoscale architectural characteristics and refractive index of cell nuclei can detect the presence of all three types of malignancy from cells labeled as "indeterminate" or "normal" by expert pathologists.

Fabrication of a Nanocoaxial Biosensor for Detection of Cancer Biomarkers

Thomas C. Chiles, Dong Cai, Lu Ren, Huaizhou Zhao, Lu Zhang, Hengzhi Wang, Yucheng Lan, Binod Rizal, Jeffrey Chuang, Michael J. Naughton, Zhifeng Ren

Boston College, Boston, Massachusetts

Metastatic dissemination of cancer cells from a primary tumor negatively impacts prognosis in patients because most conventional therapeutic interventions are limited in their success once a tumor has spread beyond the tissue of origin. Therefore, detecting cancers in the pre-malignant state is critically important, as early detection would allow for appropriate treatment modalities to be initiated prior to the onset of metastasis, thereby reducing mortality and morbidity. Biomarkers are cellular-derived material that can be used to detect disease, measure its progression, or monitor the efficacy of therapeutic intervention. Notably, clinical measurement of cancer biomarkers offers the potential for early detection of cancer disease. The research seeks to develop a novel 3-D "nanocavity" array for the detection of human cancer biomarkers. The sensor unit both constitutes a nanoscale capacitor and forms a nanoscale coaxial transmission line built around an aligned internal conductor that, in turn, can be coupled to a biomarker recognition component. The platform architecture allows for a high site density of discrete nanocoax sensors per chip (~108/cm^2) and the coaxial design facilitates impedance spectroscopy-based detection in response to biomarker recognition, thereby providing a label-free, non-optical measurement. Research in the first seven months has been directed toward optimizing nanocoax array fabrication and related electrical measurements as well as functionalizing the internal conductor for eventual protein (biomarker) recognition. We report herein the development of two routes to fabrication of coaxial nanocavity array sensors, with and without nanoporous media. The former uses a nanoporous dielectric (e.g., alumina or silicon oxide, pore size ~10 nm) in the coax annulus (or cavity), while the latter employs a non-porous dielectric that is removed via etching, leaving an empty cavity. The nanoporous device has the advantage of having large surface area exposed to target molecules, such that chemi- and/or physisorption processes can lead to enhanced molecular binding within the coaxial detection volume. The non-porous device has different advantages, including faster response time (not requiring molecular diffusion into pores) and detection of larger molecules, such as proteins. Preliminary results with each array sensor suggest detection levels on the order of sub-ppb for a variety of organic molecules. Though we are currently exploring a variety of biomarker recognition systems (e.g., antibody, apatmer), we describe recent progress on a novel molecular imprint (MI)-based protein recognition technology that relies on the electropolymerization of non-conductive polyphenol nanocoating onto the surface of CNT arrays. Ultrasensitive detection of candidate proteins was achieved via electrochemical impedance spectroscopy, including human papillomavirus E7 oncoprotein, with unprecedented sensitivity (sub-pg/L detection levels). Template protein MIs exhibited selectivity and could discriminate between conformational changes. The MI-based protein recognition holds promise for ultrasensitive measurement of minute levels of cancer biomarkers, necessary for early, pre-malignant detection. The technology can also be applicable to host of other human diseases for which valid biomarker panels are known as well as genetic cancer research for aberrant molecular pathway identification via high throughput proteomics. This research is supported by the National Institutes of Health, Innovative Technology Development for Cancer Research R21 grant.

Efficient Methods for Profiling Allele-Specific DNA Methylation in Cancer Precursor Tissues

Anupam Paliwal[1], Kristi Kerkel[1], Alexis Temkin[1], Simon Lax[1], Teresa Palomero[1], Xiaowei Chen[2], Ali Torkamani[3], Nicholas Schork[3], Benjamin Tycko[1]

[1]Columbia University, New York, New York; [2]Fox Chase Cancer Center, Philadelphia, Pennsylvania; [3]Scripps Research Institute, La Jolla, California

Allele-specific DNA methylation (ASM) is increasingly recognized as a useful epigenetic signpost for cis-acting regulatory sequences in the human genome. Thus mapping ASM genome-wide in cancer precursor tissues has the potential for identifying regulatory polymorphisms that may affect cancer susceptibility. Building on our earlier proof of principle study which used Affymetrix SNP arrays for methylation-sensitive SNP array analysis (MSNP; Kerkel K et al., *Nature Genet*, 2008) we have now successfully designed custom oligonucleotide arrays (Agilent) for finding genes with ASM. We are applying these arrays for mapping ASM with high sample throughput in multiple types of human tissues including peripheral blood leukocytes, placenta, liver, lung, and primary mammary epithelial cells. We are bringing the resulting data forward for fine-mapping of the ASM across Mb-scale chromosomal regions using high-throughput bisulfite PCR followed by massively parallel long-read sequencing (454/FLX). Technical challenges in this work include maximizing the information content of the custom arrays and achieving efficient bisulfite PCR on high-throughput microfluidics-based platforms (Fluidigm, RainDance). Using these approaches we expect to achieve a high-resolution picture of genetically determined epigenetic asymmetries in cancer precursor tissues.

DNA Methylation Profiling From Fixed Melanoma Tissues

Nancy E. Thomas, Kathleen Conway, Sharon N. Edmiston, Pamela Groben, Honglin Hao, Craig Carson, David Olilla, Haitao Chu, Pei Fen Kuan, Zakaria S. Khondker

University of North Carolina, Chapel Hill, North Carolina

Melanoma has the capacity to metastasize early and its course is rarely impacted by medical intervention. Early diagnosis is difficult due to its overlap in clinical and histologic appearance with highly prevalent benign melanocytic nevi (moles). A critical need exists for improving diagnostic methods to avoid under and over treatment of melanocytic lesions. While molecular diagnosis of melanoma holds promise, the small size of melanocytic lesions, which are typically submitted in entirety for diagnosis, requires diagnostic tests to be valid and reproducible in formalin-fixed, paraffin-embedded (FFPE) tissues. DNA methylation has emerged as a sensitive technique for assessment of occult cancer in biological samples. In this study, we investigated whether melanocytic FFPE tissues were a suitable source of DNA for high-throughput methylation array profiling and compared methylation patterns between matched pairs of FFPE and non-fixed cell lines, nevi and melanomas using the Illumina GoldenGate Cancer Panel I Methylation microarray, which interrogates the methylation status of 1505 CpG sites within promoter regions of 807 cancer-related genes.

We optimized the Illumina GoldenGate Methylation assay and validated assay performance on matched pairs of frozen and/or FFPE cell line DNA. Using >250 ng DNA, methylation profiles showed high reproducibility and correlation for frozen duplicates of 8 cell line DNAs (r^2=0.98), 20 matched FFPE and frozen cell line DNAs (r^2=0.98), and 14 FFPE duplicate DNA samples (r^2=0.97). Using admixtures of melanoma cell line (Mel-505) and peripheral blood (PB) DNA, we investigated our ability to detect the tumor methylation profile in the presence of contaminating non-tumor DNA. A "dose response curve" was performed with proportions of Mel-505 cell line DNA to PB DNA, respectively; 100/0, 90/10, 80/20, 70/30, 60/40, 50/50, 0/100. The resulting methylation profiles obtained from the pure Mel-505 cell line were highly correlated with those from Mel-505 admixtures containing between 10 and 30%

PB DNA [90/10 (r^2=0.98), 80/20 (r^2=0.94) and 70/30 (r^2=0.89)]. However, contamination with >40% PB DNA impeded our ability to detect the characteristic Mel-505 methylation profile.

Illumina methylation array profiles were evaluated in DNA isolated from 29 FFPE nevi and 22 FFPE melanomas. Histologic types of melanomas included acral lentiginous (14%), lentigo maligna (14%), nodular (9%), spindle cell (3.4%), and superficial spreading (50%). Nevi were intradermal melanocytic (38%), compound melanocytic (31%), compound melanocytic with congenital pattern (21%), and compound dysplastic with slight atypia (7%). Unsupervised hierarchical clustering on normalized methylation array data separated the total sample set into nevi and melanomas. Using class comparison analyses, 75 CpG sites in 64 genes differed significantly (with P values of <0.05) between nevi and melanomas after Bonferroni correction. Adjusted for patient age and sex, 29 CpG loci differed significantly between melanomas and nevi, including 22 hypomethylated and 7 hypermethylated CpG loci in melanoma. Using these 29 CpG sites, DNA methylation patterns distinguished melanoma from nevi, suggesting these targets could be incorporated into a diagnostic marker panel.

The results work toward the development of diagnostic methylation assays for standardizing melanoma diagnosis. In addition, knowledge of diagnostic DNA-methylations in melanoma should offer a better understanding of their involvement in melanoma development and/or maintenance and translate to the identification of new therapeutic targets.

Single-Cell Analysis Reveals Cellular Heterogeneity of Cancer and Normal Cell Lines

John F. Zhong[1], Sean Nisperos[1], Zhenyu Li[2], Sung-yung Park[3], Eric Chiou[4]

[1]University of Southern California, Los Angeles, California; [2]California Institute of Technology, Pasadena, California; [3]University of California, Los Angeles, Los Angeles, California

Cell lines especially cancer cell lines often are considered as homogeneous cell populations in characterizing cancer and in drug screening. However, recent single-cell studies indicate that individual cells from a human embryonic stem cell (hESC) colony have different gene expression profiles. These studies suggest that cellular heterogeneity is an important intrinsic character of normal and cancer cells. Large scale single-cell analysis must be performed to reveal the cellular heterogeneity of tumors from a particular cancer patient for treatment evaluation. Currently, researchers and physicians have limited capacity to perform such large scale single-cell analysis mainly due to the difficulty of manipulating small volumes of single-cells (several picoliters) and reagents. To overcome this limitation, we developed a microfluidic-based single cell analysis device to achieve the rapid separation and encapsulation of single-cells from a population of cells. With this microfluidic device, individual cells from various normal and cancer cell lines were encapsulated and manipulated for reverse transcription assay followed by real time quantitative PCR. Our results show that cellular heterogeneity is a common phenomenon in various cell lines including HeLa, 293T and normal hESC line (H9). These results underscore the limitations that are present in conventional laboratory techniques using the lysate of a population of cells to characterize and study cancer. The microfluidic device we developed provides a platform for large scale single-cell analysis in characterization of cancer cells. Individual patients may have unique cellular heterogeneity profiles in their primary tumors and circulating tumor cells (CTC) at different time points during treatment. The dynamics of heterogeneity in primary tumor and CTC during treatment can be explored for prognosis and treatment evaluation by using our single-cell analysis devices.

Developing a Single-Cell Growth Assay Platform for Monitoring Response to Cancer Therapies

Scott Manalis[1], Parag Mallick[2]

[1]Massachusetts Institute of Technology, Cambridge, Massachusetts; [2]University of Southern California, Los Angeles, California

Changes in cell growth kinetics are a hallmark of cancer cells. Anti-cancer therapies often attempt to non-specifically inhibit the growth of rapidly dividing cells by anticipating a greater impact on cancerous versus normal cells. Mechanistically, cancer cells' changes in growth patterns and rates may originate with alterations in growth signaling networks (e.g., mutations in constituent proteins, changes in protein abundances, etc.). Numerous "targeted" therapeutics are designed to halt the growth of a tumor via specific disruptions in these networks. However, an obstacle to the widespread use of targeted therapeutic approaches is their lack of generality; while highly effective in some patients, targeted agents may have little or no benefit to other patients with seemingly the same gross tumor type. For example, geftinib produces an objective response in approximately 10%-15% of patients with advanced non-small cell lung cancer.

Our studies here will be focused on developing an assay platform for measuring single cell growth with the aim of rapidly assessing the efficacy of therapeutic agents on a patient-by-patient basis. We are currently developing methods that will allow mass accumulation, volume, and fluorescence to be measured simultaneously in real time from a single cell. Therapeutic agents will be delivered either to a single cell during the measurements or to a population of cells that will subsequently be measured. Once validated, we will determine if this measurement approach can be used to classify the response of cancer cells to pathway-directed therapeutic agents.

Over the past year, we have developed a system by which a single cell can be monitored for buoyant mass changes before and after a drug treatment. The system operates in two modes: (1) a single cell is passed multiple times through the mass sensor based on the suspended microchannel resonator (SMR) and (2) the cell is captured and immobilized by a series of columns at the apex of the suspended resonator. Whereas the first mode is used to obtain a precise measurement of the cell's buoyant mass while accounting for long term fluctuations in the environment, the second is used for a fast exchange of the cell's surrounding medium allowing for drugs and stimuli to be delivered in a timely manner.

We have validated the system by measuring the growth of single mouse lymphoblast cells (L1210) before and after a treatment with 1% sodium azide. At this concentration, sodium azide will effectively block all ATP synthesis and will thus inhibit cell growth almost instantaneously. In the control experiment, we measured the growth of single mouse lymphoblast cells within its growth medium for approximately 20 minutes. We then exchanged the cells' surrounding fluid with fresh growth medium and found that the cells grew at almost exactly the same rate. In the sodium azide experiment, we also found the growth rate of the cells in media, but following this, we exchanged the cells' surrounding with media containing 1% sodium azide. We found that the cells will initially decrease in buoyant mass and eventually reach a steady state of no change in mass (as expected of a dead cell). It has been proposed that the initial decrease in buoyant mass comes from an increase in volume due to ionic imbalances which resulted from the active transport shutting down when ATP synthesis comes to a stop.

Development of a Nanoscale Calorimeter

Dale N. Larson

Draper Laboratory, Cambridge, Massachusetts

The phenomenon of extraordinary optical transmission (EOT) serves as the basis for the creation of a new approach to isothermal titration calorimetry (ITC). EOT enables the creation of extremely small, sensitive temperature sensors that can be used to determine thermodynamic properties of reacting compounds. When these sensors are placed in a grid and coupled to a microfluidics system the resulting system has the potential to reduce compound usage by 1000-fold and deliver significant improvements in sample processing throughput. Calorimetry technology is extremely valuable in the context of drug development, but conventional ITC finds very limited usage because the amount of sample required is prohibitive. The data provided enable the researcher to gain deep insight into the nature of a binding reaction.

Grant #: 1R21CA131884

Genetically Encoded Photo-Crosslinking Approaches to Map Cancer Signaling Pathways

Scott Soderling, Hirokazu Okada, Akiyoshi Uezu

Duke University, Durham, North Carolina

To appreciate how cells migrate, establish polarity, and adopt cell shape is fundamental to understanding cancer biology. Rho-GTPase signaling and tyrosine phosphorylation are two pathways that are important for these processes and they present opportunities for current and future therapeutic intervention. Unfortunately the molecular mechanisms that determine signal transduction specificities are not clear for either pathway. Our current work is to develop new proteomic screens using cellular proteins with unnatural amino acids to covalently trap protein interactions specific to these pathways in situ. Two approaches will be discussed. One approach to identify Rho-GTPase regulatory signaling complexes and another to identify phospho-tyrosine proteomes specific to subcellular compartments will be presented. Preliminary data will be shown supporting these approaches will uncover novel links between Rho GTPase and tyrosine phospho-signaling and cancer biology. Advantages and current limitations to these new techniques will be presented.

Defining Epigenetic Proteomes Using Novel Crosslinking Agents

Lucy Ann Godley, Jozef Madzo, Christopher Mariani, Elizabeth Wiltrout, Chuan He

The University of Chicago, Chicago, Illinois

Epigenetic changes alter chromatin structure, thereby regulating gene transcription. A major limitation to making significant advances in diagnosing and treating cancer based on altered DNA methylation within tumors is the fact that we do not have a thorough understanding of the mechanisms leading to abnormal DNA methylation in cancer cells. New techniques are needed to identify the DNA methyltransferase (DNMT) enzymes and their accessory proteins that mediate the DNA methylation changes of particular promoters in cancer cells. Our goal is to develop a unique method of chemical crosslinking and protein complex identification to define a quantitative molecular signature for DNA methylating complexes in cancer, using breast cancer as a model system.

We have synthesized a variety of compounds that we are exploiting for crosslinking DNMTs and accessory proteins to derivatized oligonucleotides whose sequence corresponds to promoters regulated by DNA methylation in cancer cells. Our crosslinking reagents include a cytosine with a disulfide tether on the N4-position (C*), diazirine-base derivatives that can be incorporated into the major or minor grooves of DNA to promote photo-crosslinking, and disulfide-linked deoxyribose (DLD). To date, we have achieved the most efficient crosslinking with disulfide-modified oligonucleotides containing C* and the catalytic domains of the de novo DNA methyltransferases DNMT3A and DNMT3B. The most efficient crosslinking is obtained using DNMTs with point mutation of a cysteine located in the base-flipping pocket: C710S, in the case of DNMT3A, and C651S, DNMT3B. We have achieved high efficiency crosslinking with oligonucleotides specific for the BRCA1 promoter, and are in the process of purifying the DNMT-oligonucleotide complexes and performing affinity purification of associated proteins from breast cancer cell extracts. After optimizing our approach using cultured cell lines, we will perform similar experiments using nuclear protein extracts from primary breast tumors.

Our new strategy has significant advantages over other approaches in that it assembles a protein complex directly on a specific biologically relevant DNA, which may help stabilize a protein complex that might not form otherwise. This project has the potential to transform the way we study individual genes that are regulated by DNA methylation in cancer and in other conditions in which epigenetic changes regulate gene expression. Because changes in DNA methylation represent some of the earliest molecularly defined alterations observed in cancer cells, our findings could provide the basis for new diagnostic tools to identify transformed cells based on the presence of quantitative protein levels present within DNA methylating complexes. Cancer cells could be distinguished from normal cells based on the identification of characteristic protein levels, as a signal of altered DNA methylating activity. In addition, if we are able to identify the factors involved in promoter hypermethylation in breast cancer, we could then consider developing new agents that could alter DNA methylation in a sequence-specific manner. Moreover, by stabilizing DNMT-DNA complexes through covalent crosslinking, we could facilitate structural determination of these interactions. In principle as a platform technology, our crosslinking approach can be applied to any gene of interest in any cancer.

The Biomarkers Consortium: Lessons Learned

Shawnmarie Mayrand-Chung

Office of Science Policy Analysis, Office of the Director, NIH

Abstract forthcoming.

The Science of Team Science: Why Bother With Return on Investment Analysis?

Stefano Bertuzzi

Office of Science Policy Analysis, Office of the Director, NIH

The involvement of the government in research activities is a relatively recent event in the history of the United States. Prior to 1950, the U.S. Government was essentially a nonplayer in research and development. Between World War I and World War II, most of government investments in research were in agriculture, to address the need for better nutrition. During and immediately after World War II, the government invested heavily in defense research and development activities. The U.S. Government began providing significant funding to health research in the late 1960s, triggered by the advocacy of key members of Congress, and philanthropists, such as Mary Lasker.

In this relatively short time, investments of the U.S. Government in health research have been phenomenal, and so have outcomes, which provided tremendous progress in extending the length and the quality of life of millions of people in the United States and around the world by decreasing the burden of terrible communicable and noncommunicable diseases. Several studies have attempted to measure the impact of medical research on health [1,2,3,4]. While it is often difficult to establish a clear causal relationship, it appears that the translation of biomedical research advances into practice have been key determinants of health [5]. In addition to health impact, biomedical research has also been considered an important component of economic growth and national competitiveness [6,7,8].

Policymakers recognized the relevance of biomedical research for the economy when they prominently inserted research in the "stimulus package" with the aim of jump-starting a sagging economy to deliver high returns for American taxpayers' investment. By doing so, they have also increased the pressure for accountability and transparency, providing the NIH and the other science agencies with the opportunity to develop tools and approaches to better understand the value and the performance of the sector.

This has led to the development of data infrastructures and tools that allow the collection and the analysis of a wide variety of data from different sources to inform science policy in a more rigorous manner so that the most effective policies can be implemented.

References

1. Cutler DM, Rosen AB, Vijan S. The value of medical spending in the United States, 1960-2000. N Engl J Med 355(9), 920-927, 2006.
2. Heidenreich P, McClellan M. Biomedical research and then some: the causes of technological change in heart attack treatment. In KM Murphy, RH Topel (Eds.), Measuring the Gains from Medical Research. *Chicago: The University of Chicago Press*, 2003.
3. Murphy KM, Topel RH. The value of health and longevity. *Journal of Political Economy* 114(5):871-904, 2006.
4. Lichtenberg FR. Pharmaceutical Innovation, Mortality Reduction and Economic Growth. In KM Murphy, RH Topel (Eds.), Measuring Gains from Medical Research. An Economic Approach. *Chicago: The Chicago University Press*, 2003.
5. Cutler DM, Deaton AS, Lleras-Muney A. The determinants of mortality. *NBER*, 2006.
6. FamiliesUSA. In Your Own Backyard: How NIH Funding Helps Your State's Economy. Washington, DC. Retrieved December, 2008 from http://www.familiesusa.org/issues/global-health/publications/in-your-own-backyard.html.
7. Burns LR. The Biopharmaceutical Sector's Impact on the U.S. Economy: Analysis at the National, State, and Local Levels. Washington, DC: PhRMA. Retrieved December 1st, 2009 from http://www.archstoneconsulting.com/biopharmapdf/report.pdf
8. Thursby J, Thursby M. Here or there? A survey of factors in multinational R&D location. Report to the Government-University-Industry research roundtable. Washington, DC: National Academies Press from http://books.nap.edu/openbook.php?record_id=11675&page=R1, 2006.

NIH's View of Public-Private Partnerships

Barbara B. Mittleman

Office of Science Policy Analysis, Office of the Director, NIH

Abstract forthcoming.

Evaluation of Ultra-High-Throughput qPCR Platforms for MicroRNA Profiling: Implications for Profiling Plasma MicroRNAs in Pancreatic Cancer Patients

Thomas D. Schmittgen[1], Jinmai Jiang[1], Robert Philibert[2], Megan Lerner[3], Yuriy Gusev[4], Daniel J. Brackett[3]

[1]Division of Pharmaceutics, Ohio State University, Columbus, Ohio; [2]Department of Psychiatry, University of Iowa, Iowa City, Iowa; [3]Oklahoma VA Medical Center, Oklahoma City, Oklahoma; [4]Lombardi Cancer Center, Georgetown University, Washington, D.C.

Quantitative PCR (qPCR) has become the gold standard of quantitative gene expression analysis due to the sensitivity and specificity of the PCR. A shortcoming of qPCR platforms is low throughput; typically 96 or 384 well configurations. Recently, several nanoliter scale, ultra-high-throughput instruments have been introduced. These include the Fluidigm Biomark and the Applied Biosystems Open Array. The Fluidigm Biomark and the Applied Biosystems Open Array are capable of 9,216 individual PCRs in a standard 2-hour run time. We evaluated both platforms for their ability to profile mature microRNAs (miRNAs) isolated from both tissue and plasma and compared their performance to the Applied Biosystems 7900 (384 well configuration). On tissue RNA, the mean Pearson correlation coefficient between the Biomark and Open Array was $r^2 = 0.7709$. Using an optimized protocol that included pre-amplification of the cDNA, the expression of 96 mature miRNAs was determined in plasma samples from 23 pancreatic cancer patients and healthy controls in both the Fluidigm Biomark and Applied Biosystems 7900 platforms. Of the 96 mature miRNAs profiled, only 31 were detectable in plasma. The average Pearson's correlation coefficient for the CT versus CT comparison for the individual miRNAs was quite good ($r^2 = 0.9112$). For the combined CT versus CT comparison (987 data points) the correlation was $r^2 = 0.9087$. The Î"CT to Î"CT comparison (data normalized to spike in control oligo) for the combined 31 miRNAs produced a good correlation ($r^2 = 0.8354$). The expression of 403 miRNAs and control genes was profiled in 96 patients' plasma (50 pancreatic cancer and 46 healthy controls) using the Fluidigm Biomark. Data normalization was achieved using a spike in oligo as well as with the geNorm algorithm. Hierarchical clustering of the data produced an miRNA signature that grouped a cluster of cancer patients that significantly differed from the remaining cancer patients and controls. The basis of this clustering was not due to tumor stage. These data will be mined to discover specific plasma miRNA biomarkers of pancreatic cancer that will be validated in an additional cohort of samples. We conclude that the nanoliter scale, ultra-high-throughput qPCR platforms generate data that are comparable to traditional qPCR (5 µl volume, 384 well configurations). These platforms offer a tremendous increase in throughput (increased by ~ 40-fold) and a substantial reduction in reagent cost (~10-fold).

High-Resolution Optical Molecular Cytogenetic Analysis of Fresh and Archival Tissues Using Spread Chromatin Arrays

Heinz-Ulli Weier[1], Haig Kassabian[1], Eric Lowe[1], Jingly F. Weier[1,2], Hui Zeng[1]

[1]Lawrence Berkeley National Laboratory, Berkeley, California; [2]University of San Francisco, San Francisco, California

Fluorescence in situ hybridization (FISH)-based techniques have proven indispensible for the detection of small translocations, chromosomal rearrangements or genomic imbalances in apparently normal individuals, premalignant lesions or cancer. Despite its speed and accuracy, present FISH-based assays remain limited to the simultaneous analysis of just a few, selected gene loci or metaphase spreads, if available. A thorough analysis of interphase cells is complicated by the unknown cell cycle stage of proliferating cells and the three-dimensional presentation of interphase nuclei. Recent technical developments for single cell interphase analysis address these shortfalls via chromatin fiber-based molecular cytogenetics and designer DNA probes prepared from well-characterized bacterial artificial chromosomes. As part of our efforts to translate technical progress into clinical diagnostics, we use molecular cytogenetics to study genetic changes in radiation-induced childhood thyroid cancers that arose after the

1986 nuclear accident in Chernobyl, Ukraine. The high-resolution cytogenetic analysis of interphase cells is greatly facilitated by de-condensing the interphase nuclei and spreading the chromatin on glass slides or cover slips in defined positions. We refer to such ordered arrays containing 5-20 cells per spot as "Spread Chromatin Arrays (SCAs)," and use the term "single cell arrays" when individual cells are deposited by flow sorting or mechanical micromanipulation. Interestingly, the extent of chromatin decondensation can be controlled by the volume and composition of a cell lysis buffer, while the extent and direction of the spreading is controlled by gravitational force as well as the shape and area of a reaction chamber created by hydrophobic barriers.

MMPA: A Novel Method for Simultaneous Detection of Multiple Methylated Sequences in a Large Background of Unmethylated Sequences

Baochuan Guo, X. Sun, Y. Liu, B. Shen

GLC Biotechnology, Inc., Solon, Ohio

Detecting methylated DNA derived from clinical samples is emerging as a promising non-invasive method for early cancer screening. Methods for methylation analysis of clinical specimen need to be both sensitive and specific. In addition, the methods must be of the multiplexing capability. In this SBIR project, we develop a novel method called Multiplexed Methylation Profiling Assay (MMPA), which allows for the simultaneous detection of multiple methylated sequences in a very large background of unmethytlated sequences. Specifically, we develop the MMPA assays (one for each type of cancer) for analysis of DNA methylation in colorectal, lung, and breast cancer, three deadliest cancers, respectively.

A MMPA assay consists of five steps. First, a DNA sample is treated using a bisulfite reaction converting cytosine to uracil whereas the methylated cytosine residues are unaltered. Second, the treated DNA is then amplified by the first run of PCR to generate sufficient quantities of DNA for enrichment. Third, methylated DNA is enriched using methylation-specific hybridization. Fourth, the enriched DNA is amplified by the second run of PCR to generate sufficient amounts of DNA for detection. Finally, DNA fragment analysis is used to determine the methylation status of each gene.

We have developed the MMPA assays for methylation analysis of over 50 commonly methylated genes and our result shown that the assays could readily determine the methylation status of a large number of genes in the presence of 10,000 folds more excess of unmethylated DNA. Moreover, our study demonstrated that the MMPA method is highly specific. The MMPA assays have also been successfully used to detect the presence of methylated DNA in clinical specimens such as urine, BAL, and stool.

RainDance Technology: Commercialization of Droplet Microfluidics

Darren R. Link

RainDance Technologies, Inc.

RainDance Technologies was founded in 2004 to commercialize the use of picoliter volume droplets as microreaction chambers. In 2006, a grant from the IMAT program allowed RainDance to invest in the feasibility work around the concept of droplet PCR for enrichment of target amplicons for Next-Gen Sequencing technologies. Based on this feasibility work and a subsequent round of venture financing, Sequence Enrichment was selected for productization in early 2008 and the RDT 1000 instrument was released for sale in the spring of 2009. This presentation provides an

overview of RainDance and describes the evolution of the commercialization strategy for the proprietary microdroplet technology, from the perspective of one of the company founders.

Single-Molecule Analysis and Whole Genome Amplification Technologies Based on Strand-Displacing DNA Polymerases

Paul M. Lizardi

Yale University School of Medicine

In the late 1980s and early 1990s the laboratory of Margarita Salas in Spain described the remarkable properties of a strand-displacing DNA polymerase from bacteriophage phi29. Thanks to NCI-IMAT funding, my laboratory at Yale University was able to use strand-displacing enzymes to develop novel DNA amplification technologies. In this meeting presentation I will describe the DNA amplification tools that we developed with IMAT funding. I will also talk about how the dissemination and further development of these technologies led to the commercialization of a variety of products that are greatly enabling for modern genomics research.

Rolling Circle Amplification (RCA) was developed at Yale University in starting in 1996, and it was perfected during the next few years with support from the NCI. It rapidly became an enabling component for a variety of DNA assays. Some of these assays were designed to amplify a single molecule of DNA while it remained tethered to a surface, while in other versions the DNA was free in solution. Today, RCA is an enabling component of a third-generation DNA sequencing platform developed by the company Complete Genomics. A method for whole genome amplification was developed at Yale starting in 1997, and also benefited from funding from the IMAT program. As is the case for RCA, the amplification reaction is enabled by the unique properties of strand-displacing DNA polymerases. This patented technology, known as Multiple Displacement Amplification (MDA), can generate thousands of copies of the entire human genome, starting from just a few cells. Commercial products available from Qiagen use MDA technology to facilitate biomedical research that requires the analysis of DNA starting from very small samples.

A more recent research effort at Yale, which was also funded by IMAT, focused in the development of technology for DNA methylation analysis, and the use of this epigenetic information for biomarker discovery. The DNA methylation analysis method we use employs MDA in one of the early steps, prior to the final microarray hybridization or DNA sequencing steps. We have generated whole-genome DNA methylation profiles of tumors and non-tumor adjacent tissue samples obtained from patients with head and neck squamous carcinoma. We also generated DNA methylation data using DNA from buccal epithelia of 10 normal individuals. A unique feature of this method of analysis is that it enables the interrogation of the DNA methylation levels in ALL sequence compartments of the genome, including repetitive elements. Our published results show that the younger, primate-specific members of retroelement families suffer the most dramatic loss of methylation in tumors, while the loss of methylation in nontumor margins is not global, but limited to some loci in the genome and more pronounced in specific subsets of retrotransposon families. Our most recent unpublished work involves the use of the methylation data to classify tissues in three categories, i.e., normal, margin, and tumor. Surprisingly, the information-rich repetitive DNA methylation metrics generate as good or better classification results as the metrics derived from gene promoter methylation.