University of Pennsylvania

Annual Progress Report: 2008 Formula Grant

Reporting Period

July 1, 2009 – June 30, 2010

Formula Grant Overview

The University of Pennsylvania received $8,975,120 in formula funds for the grant award period January 1, 2009 through December 31, 2012. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Molecular Mechanisms Involved in Reprogramming Cells - The reprogramming of adult cells to a pluripotent state resembling embryonic stem cells is one of the most exciting advances in stem cell biology over the past decade. These induced pluripotential stem (iPS) cells offer the potential for patient-specific regenerative therapies, new sources of cells to understand disease and for drug discovery. This project is fundamental research that will help to elucidate the process by which adult cells are converted to pluripotent cells and provide information that can be used in efforts to make the generation of iPS more efficient and safe for clinical use.

Anticipated Duration of Project

1/1/2009 - 12/31/2011

Project Overview

Induced pluripotent stem cells are the product of reprogramming somatic cells of the adult to an embryonic-like state. As with embryonic stem cells, iPS cells can be used as the starting material for generating specialized cells in regenerative medicine. However, much must be demonstrated before the potential of iPS cells in therapies can be realized. Concerns about iPS cells stem from our lack of information on the fundamental mechanism of the conversion process and how this process can be manipulated safely and effectively, that is, without introducing exogenous genes into the genome, as well as assuring that the iPS cells generated are authentic and will produce normal, specialized cells.

Specific Aim1: Determine the variables and extent of the reprogramming process at the *single* cell level
Specific Aim 2: Determine whether there are limits to the reprogramming process

Principal Investigator

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Other Participating Researchers

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Expected Research Outcomes and Benefits

Strategies in regenerative medicine to develop therapies for diseased, damaged and worn out body parts will rely heavily on stem cells. Ideally, these stem cells must match the patient or they will be rejected by the body's immune system. Induced Pluripotential Stem (iPS) cells offer the potential for patient-specific regenerative therapies, new sources of cells to understand disease and for drug discovery. Despite their immense promise, iPS cells face several hurdles on the road to clinical applications. If this research is successful, the efficiency for generating patient-specific iPS will be increased, the cells derived from iPS for therapies will be more effective and concerns over the safety of the cells will be alleviated. This project addresses fundamental biologic mechanisms involved in the process by which adult cells are converted to the pluripotent state that must be understood so that we can address the issues of efficiency and safety as well as determine the real utility of the iPS cells in effecting therapies.

Summary of Research Completed

Aim1: Determine the variables and extent of the reprogramming process at the single cell level

We cloned the pluripotency factors Oct4, Sox2, Klf4, and Myc into the plasmid pEF-DEST51 using Gateway technology (Invitrogen Corporation). The resulting plasmids contained each of the pluripotency factors under the control of the T7 bacterial promoter, with a C-terminal V5 tag on each protein. In order to validate the T7-driven expression of the factors, we performed in vitro transcription and translation using the TnT® T7 Coupled Wheat Germ Extract System (Promega). We performed a Western blot for V5 on the resulting protein, and all four constructs expressed V5-tagged protein at the appropriate molecular weights (Figure 1).

Messenger RNA (mRNA) for each pluripotency factor was transcribed from the pEF-DEST51 plasmids using T7 RNA Polymerase. Purified mRNA was then phototransfected into primary mouse embryonic fibroblasts (PMEFs). We phototransfected approximately 100 PMEFs over the course of three experiments. Phototransfected cells were maintained in mouse embryonic stem cell medium for up to three weeks. No embryonic stem cell-like colonies were observed in any experiments.

In addition to phototransfection, we generated lentiviral vectors to express pluripotency factors in collaboration with the Vector Core at the University of Pennsylvania. Using the pLOVE lentiviral backbone, we generated viruses for the expression of Oct4, Sox2, Klf4, and Myc, as well as green fluorescent protein. The Vector Core generated concentrated, high titer stocks of these viruses. We confirmed that these viruses can generate induced pluripotent stem (iPS) cells from PMEFs, as measured by colony formation and alkaline phosphatase staining. These vectors, shown in Table 1, are now readily available for use by all members of the Institute for Regenerative Medicine at the University of Pennsylvania.

Aim 2: Determine whether there are limits to the reprogramming process

The goal of this research is to determine the capacity of known maternal-effect genes and oocyte-specific maternal genes encoding putative transcription factors to reprogram mouse embryonic stem cells to 2-cell blastomeres.

Research Design: We have identified two transcripts, expressed specifically at the 2 – 8 cell stage in mouse pre-implantation embryos to be used as potential markers for this stage of development. Erv4/MuERV-L is a retrotransposon specifically expressed in pre-implantation embryos. We performed quantitative RT-PCR analysis to determine the relative expression of Erv4 in preimplantation embryos as well as mouse ESCs (Figure 2). Erv4 is highly expressed in 2-cell embryos with reduced expression by the 8-cell stage. Importantly, Erv4 is not detected in the blastocyst or in the mESC lines analyzed, making Erv4 an ideal marker of the 2-cell stage embryo. We have generated a reporter and selection construct containing a GFP and hygromycin fusion gene flanked by the Erv4 LTRs, which will enable identification and selection of cells with putative blastomere characteristics following treatment with lentiviral vectors containing the candidate reprogramming factors described below. We will create a line of stably transfected mESCs containing this construct and will use this line of mESCs to generate a transgenic mouse line carrying the Erv4 reporter.

A second marker of cleavage-stage embryos is oocyte-specific homeobox (Obox6), a transcript expressed only in 2 to 8-cell embryos with expression peaking in the 8-cell embryo (Figure 3). Through qRT-PCR analysis we determined the Obox6 is not expressed in the blastocyst or in mESCs lines analyzed. The genomic structure of Obox6 has not been characterized, therefore we are generating a GFP knock-in at the Obox6 locus on an Obox6-containing BAC (Roswell Park, RP23-116J), utilizing BAC recombineering to insert a GFP – Hygromycin cassette into the first intron of Obox6 on RP23-116J. The 'marked' BAC will be injected into pro-nuclei of 1-cell embryos. GFP positive embryos will be cultured 96 hours and mESCs will be derived from the resultant blastocysts. This method will enable us to evaluate the appropriate expression of GFP from the transgenic Obox6 locus and will allow us to generate mESCs directly from embryos expressing the transgene appropriately. The mESCs derived in this manner can be evaluated for stable integration of the transgene by a loxP flanked cassette containing the neomycin resistance gene driven by the PGK promoter inserted on the BAC 3' of the Obox6 locus, which can subsequently be excised by Cre recombinase if desired.

To induce blastomere-like cells, we will transduce the reporter mESCs described above with combinations of mRNAs of the following factors sub-cloned into the lentiviral vector pLOVE

(lentiviral over-expression). For these experiments we have chosen to work with maternal-effect genes, i.e., genes that are not required for oocyte development but are required for events following fertilization, are ideal candidate genes for driving the changes of gene expression during the maternal to zygotic transition. The function of these proteins ranges from transcription factors, to chromatin modifiers, to epigenetic modifiers and members of the subcortical maternal complex, this list includes Hsf1, Npm2, Zar1, Pms2, Stella, Dnmt1o, Dnmt3a, Dnmt3l, Ctcf, Sebox, Brg1, Bnc1, Brwd1, Ube2a, Mater, Floped, Padi6, and Tle6. We have sub-cloned cDNAs representing each of these proteins into the lentiviral vector pLOVE to transduce combinations into mESCs to induce formation of blastomere-like cells determined by expression of the marker described above. We have chosen lentiviral vectors both because of our experience utilizing them for gene transfer as well as for their ability to integrate into the host genome and thereby enabling prolonged expression in transduced cells.

We performed transductions of factors singly to both mESCs (non-transgenic) and HEK 293T's to test the viral integration and protein expression of candidate factors by western blot. The HEK293T's used as a control showed protein expression at the correct size for each factor, however, the mESCs did not express any proteins detectable by western blot, nor was GFP expressed when cells were transduced with pLOVE-GFP. Subsequently, we performed transductions with lentiviral vectors containing GFP driven by a variety of promoters to determine a promoter, which could be used to drive factor expression in mESCs and found that the EF1a promoter is sufficient to drive expression in mESCs. Currently, we are subcloning the candidate reprogramming factors to the lentiviral vector, pLEF to be used in mESC reprogramming experiments. We will then begin reprogramming experiments in mESCs. After transduction, we will optimize timepoints for media changes to take into account the different energy requirements of cleavage-stage blastomeres compared to mESCs. The eGFP-positive cells will then be physically isolated by micromanipulation for further analysis as described below.

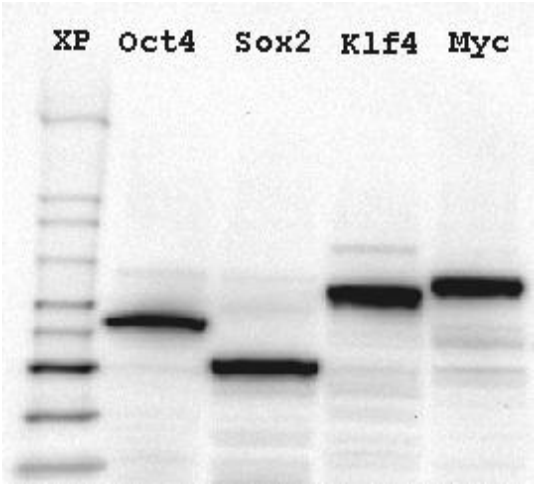

Figure 1. Western blot for V5 tag on each pluripotency factor. XP: MagicMark XP protein size standard (Invitrogen)

Lot #	Vector Name	Average Infectious Titer
Y341	VSVG.LOVE.cPPT.CMV.mKlf4	1.88e09
Y345	VSVG.HIV.LOVE.cPPT.CMV.GFP	1.57e09
Y347	VSVG..LOVE.cPPT.CMV.mMyc	1.26e08
Y348	VSVG..LOVE.cPPT.CMV.mOct4	1.24e08
Y351	VSVG.HIV.LOVE.cPPT.CMV.mSox2	pending

Table 1. Lentiviral vectors for the expression of pluirpotency factors.

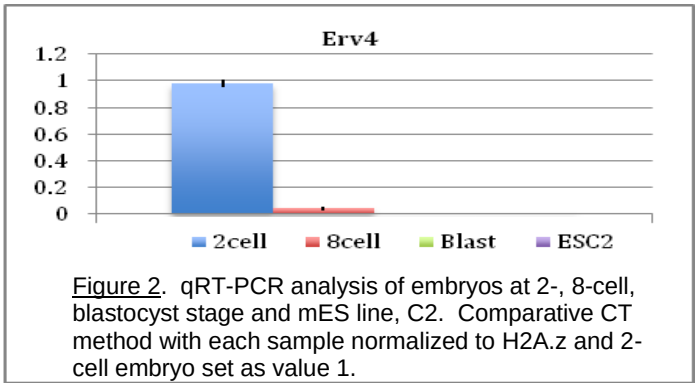

Figure 2. qRT-PCR analysis of embryos at 2-, 8-cell, blastocyst stage and mES line, C2. Comparative CT method with each sample normalized to H2A.z and 2-cell embryo set as value 1.

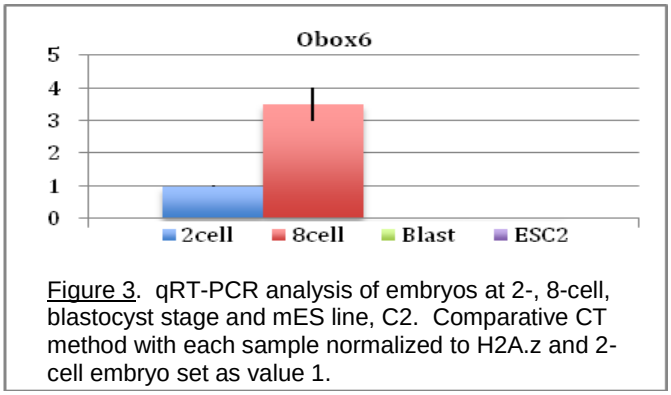

Figure 3. qRT-PCR analysis of embryos at 2-, 8-cell, blastocyst stage and mES line, C2. Comparative CT method with each sample normalized to H2A.z and 2-cell embryo set as value 1.

Research Infrastructure Project 2: Project Title and Purpose

Nanofabrication Lab for Biosensors and Biomeasurements – Research Infrastructure - This research infrastructure project renovates Penn's Wolf Nanofabrication Facility, expanding the cleanroom (filtered air) space which is required for micron and nanoscale technology and medical research. This facility addresses key needs that are shared by Penn, the NIH Roadmap, and the Commonwealth of Pennsylvania: it provides tooling that allows test beds to be fabricated to measure properties of cells and to create devices which can change them. Renovation of space in the Moore Building is essential to Penn's efforts to create an interactive environment that both

promotes the research initiatives and enables the recruitment of new faculty with expertise in micromedical devices. With these new collaborators and resources, Penn's researchers will create micron and nanoscale biomedical devices for the benefit of the citizens of the Commonwealth.

Anticipated Duration of Project

1/1/2009 – 10/1/2010

Project Overview

This project enables researchers to build devices for sensing and measuring biological systems from the cellular level down to the molecular level. Building any device at the micron or nanometer scale requires high performance equipment and an environment free of contaminants. Typical electronic devices are not biocompatible and cannot be implanted. Biocompatible devices need special coatings and surface treatments. Adding this capability requires more highly clean space for tools as well as the utilities for operating the tools.

Building one device may require multiple, different fabrication tools. Since the tools are expensive and complicated, they are housed in a shared facility with professional staff. The facility is used by Penn researchers from Engineering, Arts & Sciences and Medicine, with occasional use by researchers from Drexel University, Fox Chase Cancer Center and start-up companies. After the renovation project is over, we plan to help cover the maintenance costs of the lab by offering use of the facility to more non-Penn users for fees which are far less than what it would take for researchers to obtain the tools individually. We will also add more Penn users. This will enable more researchers in the Commonwealth to develop state-of-the-art techniques and patentable devices for biomedical research.

The facility is 3248 sf, of which 1288 sf is the highly clean space required for state-of-the-art fabrication tools. This project converts 670 sf to become highly clean space, which is more than a 50% increase. It also converts 284 sf to a safe and secure storage area for hazardous chemicals and gases, installs scrubbers for safe discharge of exhaust gases and improves lab utilities such as a nitrogen system and hazardous gas monitoring system.

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None

Expected Research Outcomes and Benefits

Nanotechnology includes a wide range of technologies that measure, manipulate or incorporate materials and/or features with at least one dimension between 1 and 100 nanometers (nm). At these scales reside the basic biology, chemistry and physics that govern chemical and biochemical reactions. Understanding what happens inside cells is critical to prevention, detection and treatment of disease. Experiments in nanotechnology are done inside wet laboratories with chemical fume hoods and in spaces designed to accommodate multiple large pieces of complex, sensitive equipment. Such facilities rank among the most expensive structures a university can construct.

Improvements to the Wolf Nanofabrication Facility, including a 50% expansion of the cleanroom space, will enable researchers to do many different types of experiments in the future. One of the first devices which will be built using the Facility will be a prostate cancer detector, built from carbon nanotube transistors. Another device will be a sensor which will be used to map biological and chemical signals in the brain, using nanowires. This will provide a vital platform to understanding the fundamental origins and causes of neurological diseases, ultimately aimed at effective prevention and long-term treatment. Other nanowire devices will be built to detect individual molecules which are indicators of disease. These and many other experiments will be conducted at the University of Pennsylvania, leading to research publications and patents for medical devices of the future.

Summary of Research Completed

With the exception of project close-out and air particle count for the clean room, the lab improvement work and tool installations were complete by 6/30/2010. It is already in active use for research.

This project expanded and improved the Wolf Nanofabrication Facility (WNF). It installed scrubbers for safe discharge of exhaust gases and improved lab utilities such as the nitrogen system, chilled water system, compressed air system, hazardous gas monitoring system, visible and audible alarms, additional electric service and Very Early Smoke Detection Apparatus (VESDA). 670 sf was converted to become highly clean space, more than a 50% increase in cleanroom size. Walls were painted with cleanroom paint, ceiling tiles were replaced with cleanroom tiles, obsolete utilities were removed, air filters were added and the whole area was cleaned. 284 sf was converted to a safe and secure storage area for hazardous chemicals and gases, housing all gas cabinets. New tools were installed in the new cleanroom space, including electric hookup and piping of gases from the chemical storage area to the new space.

WNF Construction Milestones in FY2010

- 8/17/09 tool installation work substantially complete

- 9/24/09 construction work complete, including Very Early Smoke Detection Apparatus (VESDA) and fire alarm systems
- 11/4/09 YES Oven, Atomic Layer Deposition, and Aluminum Nitride tools commissioned and in use
- 12/23/09 Revamped Process Chilled Water system up and running
- 1/26/10 Reactive Ion Etcher and Plasma Enhanced Chemical Vapor Deposition tools commissioned and in use

Research Supported by the Construction Project

The WNF is used for the fabrication of custom devices for biomedical research experiments. For example, R. Jannat, D. Hammer *et al.* studied the effect of underlying tissue mechanics on neutrophil adhesion and migration using devices created in this facility. They quantified neutrophil migration and traction stresses on compliant hydrogel substrates with varying elasticity in a micromachined gradient chamber in which they could apply either a uniform concentration or a precise gradient of the bacterial chemoattractant formylpeptide. Their findings, as reported in "Neutrophil adhesion and chemotaxis depend on substrate mechanics," 2010 *J. Phys.: Condens. Matter* 22 194117, can be used to guide the design of migration inhibitors that more efficiently target inflammation.

A custom device was created for this experiment using photolithography in a series of steps shown in Figure 1. The steps correspond to specific tools and processes in the WNF. A negative master template was prepared by depositing SU8 photoresist on a 3 inch silicon wafer, then exposing to ultraviolet light through a high resolution negative transparency mask in a Karl Suss mask aligner. After exposure and development, they had a raised network of channels on the surface of the wafer which could be used to make multiple castings. Each casting was created from a mixture of polydimethylsiloxane (PDMS) and a cross-linking agent, was cured in an oven and was peeled away from the silicon wafer. In the final step, the casting was sealed to the glass coverslip containing immobilized hydrogels using brief exposure to reactive oxygen plasma under vacuum. The results were precisely identical gradient generators, each used only once in an experiment. The experiments, including isolation of neutrophils from whole blood obtained from healthy human donors, were conducted in other labs.

Prototypes of flexible and stretchable organic semiconductor devices for active physiological sensing and stimulation are being used to map the sensory cortex and epileptic networks in rats with pilocarpine and pigs with cardiac arrhythmias. They are pentacene thin film transistors on Kapton® flexible plastic substrates as shown in Figure 2. This work by C. R. Kagan and B. Litt has made significant progress in the last year, including the patent applications "Self-adaptive bio-signal sensing and modulation device," C. R. Kagan, B. Litt, J. Viventi, filed, May, 2010 [provisional May, 2009] and "Organic Semiconductors Capable of Ambipolar Transport," C. R. Kagan, S. R. Saudari, filed, December, 2009 [provisional December, 2008]. These flexible, stretchable devices are created using the photolithography tools and the parylene coater in WNF. Other layers of the devices are made in the labs of individual investigators. The devices are also characterized and tested *in vivo* in normal animals and animal models of human disease.

Parylene's properties such as chemical resistance, low dielectric constant and dissipation factor,

biocompatibility and low permeability to gases and water make it very attractive for medical research. Parylene is the barrier between semiconductor materials and living cells, enabling creation of highly sensitive implantable devices. The Specialty Coating Systems 2010 Labcoter installed in WNF as part of this project is used to deposit highly conformal, uniform, and pinhole-free films at room temperature thus avoiding many of the issues encountered in high temperature deposition methods. This is especially valuable for flexible and stretchable devices, which would be damaged by heat.

A.T. Charlie Johnson and the Penn Team on the DARPA Real Nose Project are working to develop a class of nano-enabled, biomimetic vapor sensors appropriate for future use in an "electronic nose" system. The sensor concept is to integrate biological mammalian olfactory receptor proteins with electronic carbon nanotube transistors, so that the nanotube device would "readout" the olfactory receptor activity and transduce it into an electrical signal, and in this way replace the cellular signaling pathways that perform this task *in vivo.* It should be noted that olfactory receptors are members of the G-protein coupled receptor family of transmembrane receptors. G protein-coupled receptors are involved in many diseases, and are also the target of approximately 30% of all modern medicinal drugs. The results described below thus should generalize to this broader class of medically important molecules.

The production of the sensors required use of lithographic, thin-film deposition, and other nanofabrication tooling included in the WNF. Sensors were fabricated using three different mammalian olfactory receptor proteins that were expressed in Sf9 insect cells by the Brand group (Monell Chemical Senses Center, Philadelphia) and then purified at Penn. The sensors were tested against a panel of 10 analytes, and sensor responses were compared to tests conducted using the same analytes and the same set of olfactory receptors expressed in a biological surrogate system. As seen in the Figure 3, the responses of the receptor-functionalized nanotube devices were identical to those of the biological surrogate system in almost all cases tested, the first time to our knowledge that any membrane protein has been interfaced to a nanoscale device for signal transduction. This work has been submitted to *Nature*.

It would be inefficient for each lab to have separate tools to build custom research devices, because the tools are complex, sensitive and expensive. Typically these tools are a small part of a much larger, complex protocol. They are used intermittently by researchers, which makes a shared facility cost-effective. Some groups of tools have additional special needs. For example, the photolithography area of WNF has yellow light due to the photosensitive chemicals in the etching process, as shown in Figure 4. These tools need to be in a clean room where dust and particles are kept out of the air. Based on preliminary results, WNF is expected to be rated as a Class 1000 clean room, which is a significant improvement. The clean space is also 50% larger. Chemicals and gases used in the processes include toxics, solvents and pyrophorics. However, since the air is heavily filtered, it moves too fast for standard smoke detectors, requiring a VESDA. Special lighting, clean space and non-standard smoke detectors are provided in WNF to avoid needing that expense in individual research labs.

WNF is shared by many researchers who use the same tools but work on a wide variety of projects. Over 100 researchers have used the lab and benefitted from the improvements in

January-June 2010. Improvements to the facility have increased the quality and quantity of research results.

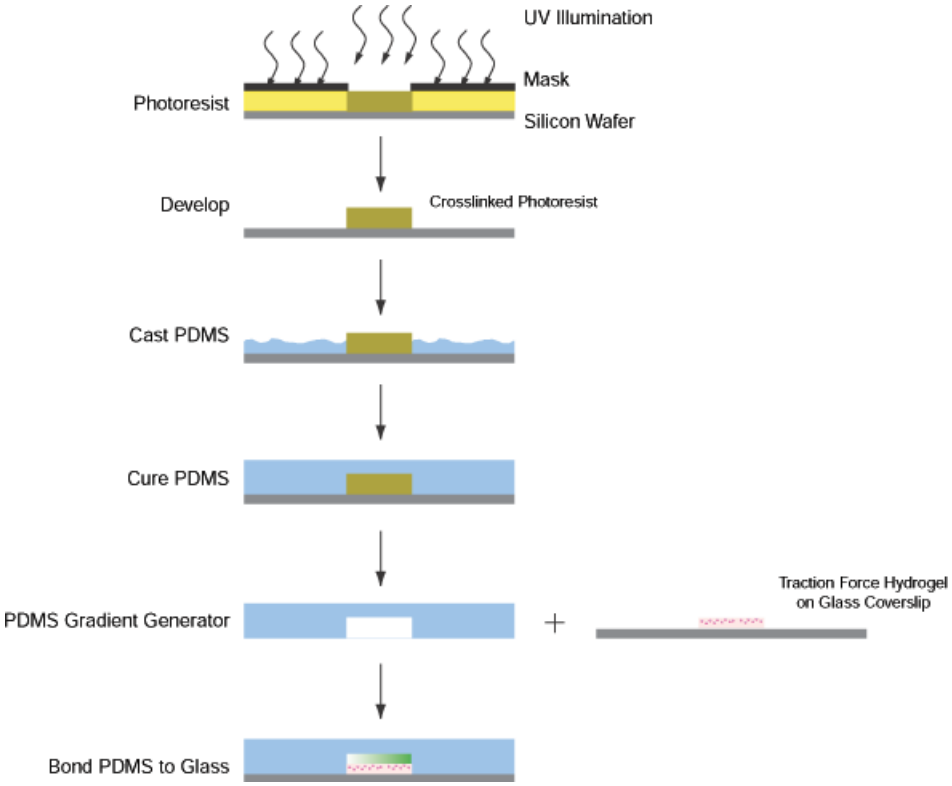


Figure 1: Fabrication of a microfluidic system to study neutrophil responses to gradients on hydrogel substrates

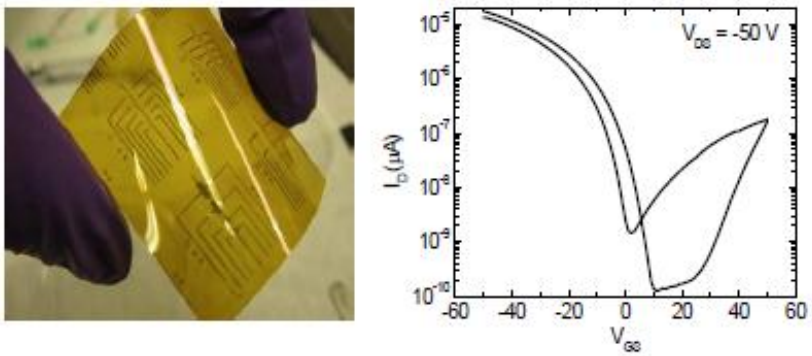
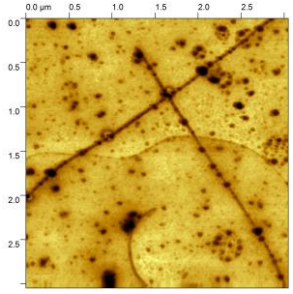
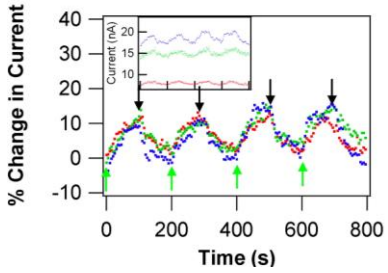


Figure 2: Ambipolar, bottom contact pentacene transistors fabricated on Kapton® and their device characteristics.

A B C





	mOR174-9	mOR203-1	mOR256-17
eugenol	D F		
2-heptanone			
heptanal			
acetophenone			
cyclohexanone			
2,4-DNT			
amyl acetate		N/T	
methyl benzoate		N/T	

Figure 3: A: Atomic force microscope image showing carbon nanotubes functionalized with olfactory receptor molecules. B: Sensor responses to the compound eugenol (a component of clove oil) of a nanotube transistor functionalized with mouse olfactory receptor mOR174-9. Eugenol exposure starts at the times indicated by green arrows, and the sample is flushed with clean air 100 seconds later (black arrows). The overlay of the red, green, and blue data sets indicates that responses are stable in time and between devices. C: Table showing responses of three different ORs to a panel of 8 distinct analytes. Green indicates a strong response, red little or no response, and N/T that the combination was not tested. For each OR/analyte pair, the left box indicates the device response ("D"), and the right box indicates the response of the biological surrogate, a frog egg ("F"). The device and surrogate responses agree in 18/22 tested combinations.

Figure 4**:** Xenon Difluoride Silicon Etcher in photolithography room

Research Project 3: Project Title and Purpose

Effects of Nicotine on Mu Opioid Receptor Binding - We will evaluate whether there are different effects of nicotine on mu opioid receptors in smokers with different genetic backgrounds. This will help us to understand the brain related mechanisms that explain why

smokers with a particular gene variant for this brain receptor are at higher risk for smoking relapse.

Anticipated Duration of Project

1/1/2009 - 12/31/2011

Project Overview

Broad Research Objective: To characterize the brain mechanisms that explain the association of the mu opioid receptor (OPRM1) gene Asn40Asp polymorphism association with nicotine dependence.

Specific Aim and Hypotheses: To examine the effects of IV nicotine (vs. placebo) on mu opioid receptor binding potential (MOR BP) in smokers stratified by *OPRM1* genotype.

> Hypothesis 1: Compared to smokers homozygous for the Asn40 allele (high risk), those carrying the Asp40 allele (low risk) will exhibit attenuated effects of nicotine on MOR BP in ventral striatum. Specifically, in the placebo session, the Asn40 group will have higher MOR BP (increased availability of MORs) than the Asp40 group; in the nicotine session, MOR BP should decrease more in the Asn40 group than the Asp40 group (due to greater endogenous MOR neurotransmission).
>
> Hypothesis 2: Asp40 carriers will have attenuated subjective responses to IV nicotine (e.g., "drug liking").

Research Design and Methods (revised July 2010): This human positron emission tomography study will assess effects of nicotine on mu opioid receptor (MOR) binding potential in 24 chronic smokers stratified by *OPRM1* A118G genotype (12 AA and 12 AG/GG). In the first experiment, smokers will participate in two PET imaging sessions with [^{11}C]Carfentanil after overnight abstinence: (a) after smoking a nicotine containing cigarette, and (b) after smoking a denicotinized cigarette. The primary outcome variable is MOR binding potential (BP) (reflecting receptor availability) in ventral striatum and additional regions of interest (ROIs). In addition, we will complete a single PET imaging session with 20 nonsmoker controls (10 of each genotype).

In the second experiment, we will follow the identical procedures, except that nicotine will be administered intravenously: (a) IV nicotine 1.0mg/70kg; and (b) IV placebo (saline) (within-subject, double-blind, counter-balanced order). The combination of neuroimaging and genetics in these two experiments, focusing on different modes of nicotine delivery, will elucidate the functional significance of the Asp40 variant and its role in the neurobiology of nicotine dependence.

Principal Investigator

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Other Participating Researchers

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Expected Research Outcomes and Benefits

Genetic association studies in psychiatry and addiction are plagued by nonreplications. However, there is a critical mass of positive studies linking the OPRM1 Asn40Asp variant with smoking behavior and relapse. Because nicotine dependence is a complex trait, investigation of in vivo brain imaging data may provide a stronger genetic signal than self-reported behavioral measures used in human genetic association studies. The proposed project will clarify the role of *OPRM1* Asn40Asp in nicotine dependence using in vivo imaging of nicotine effects. This research will provide valuable information to increase our understanding of the neurobiology of nicotine addiction and aid in the development of new medications for this significant public health problem.

Summary of Research Completed

Sample: 24 smokers of European ancestry (12 AA and 12 AG/GG, matched for sex) and 20 non-smoker controls (10 AA and 10 AG).

Methods: The smokers completed two 60-minute [^{11}C]carfentanil PET scans following overnight abstinence from smoking. Prior to each PET scan, they smoked either a nicotine (0.6 mg) cigarette or a denicotinized (0.05 mg) cigarette, using a standardized puffing procedure. Non-smokers completed a single 60-minute [^{11}C]carfentanil PET scan.

Analysis: MOR binding potential (BP_{ND} [$_{ND}$ = non-displaceable, specific binding]) was the primary outcome. PET images were motion-corrected by aligning each subsequent 10 min frame to the first 0-10 min PET frame using Statistical Parmametric Mapping (SPM5) software (http://www.fil.ion.ucl.ac.uk/spm). A mean realigned image was also generated. Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer 2002) was used to define the Occipital Region of Interest mask which was coregistered to the subject space using SPM5 using the mean realigned image. The 30-50 minute realigned frames were averaged and transformed into BP ratio maps using the mean from the occipital mask (specific binding voxel/occipital cortex [reference]-1). The BP ratio maps were then normalized to the standardized Montreal Neurological Institute (MNI) coordinate system using default parameters and the PET template provided by SPM5. Normalized BP images were then smoothed with a Gaussian kernel (fwhm=8mm) and input into a whole brain voxel-wise analysis. One control and one smoker were excluded for poor quality of data.

To test for effect of Genotype in Controls, a whole brain two sample T test of the AA and *G groups was performed and Tmaps contrasting the two groups were generated. Similarly to test for effect of Genotype in Smokers, a whole brain T test of the AA and *G allele smokers was performed for the Nicotine Session and contrast maps were generated. Resulting T maps adjusted for multiple testing using a cluster wise correction at p=0.01. In addition, a 2 Group (AA vs *G Genotype) by 2 Session (nicotine vs. denicotinized cigarette) whole brain voxel-wise anova in smokers tested for exploratory interaction effects between genotype and session. The interaction F map was uncorrected at p=0.05 only including clusters greater than 100.

Results: Several regions of reduced BP were identified in the *G versus the AA groups in both healthy (nonsmoker) controls (e.g., anterior cingulate cortex, middle temporal gyrus and putamen) and in the smokers (e.g., anterior cingulate cortex, middle temporal gyrus, cingulate gyrus and insula). In the smokers, genotype x session effects were observed in middle frontal gyrus, superior temporal gyrus, and cingulate gyrus.

Plans: Having completed accrual for this first experiment focused on nicotine delivery via cigarette smoking, we have initiated the second experiment to examine the effects of intravenous nicotine versus saline on MOR BP in 24 smokers (12 AA and 12 AG/GG). To date, we have enrolled 15 eligible smokers into the study (9 AA and 6 AG).

Research Project 4: Project Title and Purpose

Validation of Imaging Markers for Use in Cancer Clinical Trials - Biomarkers to identify appropriate patient populations and detect early response represent a significant opportunity to bring cancer drugs to the clinic more expeditiously and at a reduced cost. Advances in medical imaging already can provide quantitative in vivo assays of tumor anatomy and function in cancer patients. Support of quantitative imaging in cancer clinical trials has been limited by the lack of coordinated efforts between clinical imaging specialists and clinical cancer researchers. This project will establish a collaboration between the oncology investigators engaged in clinical trials of cancer therapy and imaging investigators to develop and validate imaging biomarkers to predict and monitor the response to therapy. This will impact our ability to develop and evaluate new therapies for cancer.

Duration of Project

1/1/2009 - 6/30/2010

Project Overview

1. Develop an infrastructure to support standardized analysis of images performed on cancer trials at Penn: The project will develop an operational interface between the cancer center and the radiology department. The approach will employ a dedicated imaging research coordinator with experience in imaging and image management who will coordinate management of imaging data resulting from cancer trials such that they are made available in a centralized 3D imaging laboratory for analysis and interpretation. A cadre of imaging clinical scientists will oversee the analysis according to standardized protocols. This will have the effect of reducing variability in

the determination of such endpoints as progression free survival and improve the performance of cancer trials across the Abramson Comprehensive Cancer center.

2. Explore novel approaches to image analysis to improve imaging markers for cancer trials: The extraction of quantitative data from imaging examinations is a critical component of the imaging clinical trial. This activity may include simple geometric measurements made by radiologists, complex reader assessments, semi automated and automated quantitative analysis which may also include complex modeling. Currently, response and progression on cancer trials is evaluated by measurement of tumor size and assessing growth and shrinking. This analysis does not account for scenarios such as tumor necrosis without significant changes in tumor size, or reduced tumor proliferation. As part of this project, novel approaches to image analysis such as adjusting size for percent necrosis (identified by the absence of contrast enhancement) or dynamic MRI perfusion measurements will be explored. This will involve performing these analyses on patients on trial, and correlating the results against patient outcome (survival, disease free survival). It is anticipated these novel approaches will better correlate with outcome and reduce the number of patients needed to evaluate treatments in future trials.

Principal Investigator

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Other Participating Researchers

Daniel Pryma, MD, Chatain Divgi, MD, Mitchell Schnall, MD, PhD, Sharyn Katz, MD, Laurie Loevner, MD, Drew Torigian, MD – employed by University of Pennsylvania

Expected Research Outcomes and Benefits

1. More effective clinical cancer trials at the Abramson Cancer Center: As a result of the coordination and standardization of image analysis, it is anticipated that the data derived from imaging studies will have reduced variability. This will increase the power of trials that utilize imaging endpoints such as progression free survival and response rate. The improvement of effectiveness of the trials utilizing current endpoint will impact a wide range of clinical research performed at the Abramson Cancer Center.
2. Clinical trials based on new imaging marker: The exploration of newer image analysis methods that result in new markers will result in improved endpoint for clinical trials. This is anticipated to result in trial endpoints that will detect response/progression earlier in the course of treatment resulting in quicker trials. This will have a broad impact on the development of newer therapeutics by reducing the clinical trial timeline.

Summary of Research Completed

Aim 1: Integration of appropriate imaging endpoints into cancer clinical trials performed at Penn

A clinical imaging core was formally established within the Abramson Cancer Center. The core is co-directed by Mark Rosen, MD, PhD and Chaitan Divgi, MD. Imaging liasons to the various research programs within the Cancer Center are being developed. The program assignments include:

Breast: Mitchell Schnall: MD, PhD
Radiobiology and Imaging: Chaitan Divgi, MD
Clinical Investigations: Mark Rosen, MD, PhD
Tobacco and Environmental Carcinogenesis: Sharyn Katz, MD

A formal imaging review is now performed as part of the Cancer Center scientific review of all clinical research protocols.

Aim 2: Oversight of conduct and analysis of imaging performed under cancer clinical trials

Two positions were created to support implementation of imaging in cancer center clinical trials. An analysis technologist was hired (Scott Steingall) to oversee image analysis and data management. The imaging clinical trials operations manager (Kathleen Thomas) oversees the development of scheduling, billing, protocol monitoring and implementation of protocols. A software package to support RECIST analysis was developed within the application development environment of the TERA INTUITION software package. To date, 20 separate trials have used the image analysis services. 189 imaging endpoints have been analyzed in 41 subjects.

Aim 3 Develop Novel Approaches to image analysis to improve imaging markers for cancer trials:

Methodology for streamlined processing and analysis of DCE-MRI data have been developed under the guidance of Dr. Rosen and Sarah Englander, PhD, technical director of the clinical imaging core. Currently, there are five active clinical trials in the Cancer Center using DCE-MRI to evaluate tumor responses to anti-vascular therapy. As the DCE-MRI method uses novel radial (back-projection) MRI data acquisition with off-line processing, off-line analytic tools are required. Given the growing volume of DCE-MRI evaluation at Penn (nearly 300 DCE-MRI imaging studies over ten clinical trials in the past five years), there is a need to implement more efficient processes to allow for the tumor and vascular segmentation required for DCE-MRI analysis.

To this end, image visualization and segmentation software has been written using IDL image processing software. This process allows the radiologist to view the radial image sets (sub-aperature, KWIC processed, or full-aperature) to delineate tumor and vessel boundaries. The radiologists segmentation is saved in a fully-retrievable set of data files that includes reader ID and timestamp of the analysis to facilitate kinetic modeling of tumor vascular function, and allow

for intra- and inter-reader reproducibility studies. Additional analytic tools for kinetic modeling have been developed in conjunction with the research laboratory of Hee Kwon Song, PhD in the Radiology Department at Penn. These tools facilitate the standardization of MRI signal intensities through improved methods for baseline correction of pre-gadolinium tumor and vascular signal intensity, as well as corrections for actual flip angle measurements built into more recent imaging protocols.

Research Project 5: Project Title and Purpose

Understanding the Biology of Residual Neoplastic Disease - A cardinal feature of human breast cancers is the survival and persistence of residual neoplastic cells in a presumed quiescent state following the apparently successful treatment of the initial tumor. Ultimately, these residual cells re-emerge from their dormant state and resume growth, leading to cancer recurrence. Indeed, analyses of bone marrow samples show that disseminated cells are present in 20-40% of primary breast cancer patients without any clinical or histopathological signs of metastasis. As such, residual neoplastic disease is a major obstacle to the successful treatment of breast cancer and other human cancers. Accordingly, the purpose of this project is to investigate the biological properties of residual neoplastic cells, since these cells represent the reservoir from which tumor recurrences invariably arise.

Anticipated Duration of Project

1/1/2009 - 12/31/2010

Project Overview

A particular difficulty in understanding the mechanisms of breast cancer recurrence has been the challenge of identifying and isolating residual neoplastic cells in patients, and the lack of animal models that recapitulate this key feature of breast cancer progression. We have generated conditional transgenic mouse models for breast cancer and have obtained important preliminary evidence indicating that essentially all oncogene-induced mammary tumors that have regressed to a non-palpable state following oncogene down-regulation leave behind residual neoplastic cells. These represent a unique set of genetically engineered models for residual neoplastic disease in breast cancer induced by defined oncogenic pathways. In this project, we propose to use these models to define critical features of residual neoplastic disease relevant to developing improved therapeutic approaches to human cancers.

The specific aims of this project are: Aim I: Identify morphological lesions containing residual neoplastic cells; Aim II: Analyze the cellular components of residual neoplastic lesions; Aim III: Determine if autophagy is a survival mechanism for residual neoplastic cells. These aims will be accomplished by using doxycycline-inducible transgenic mouse models in which oncogenic pathways important in human cancer can be conditionally activated or inactivated. Following tumor development in these models, tumor regression can be induced by oncogene down-regulation in a manner that simulates the treatment of patients with molecularly targeted agents. The tumor cells that survive oncogene down-regulation will first be identified in the mammary glands of these animals by histopathological and cell marking approaches. The

cellular components and cellular microenvironment of these residual neoplastic cells will then be defined by co-localization of cell-type specific markers. Finally, the role of autophagy in the survival of residual neoplastic cells will be determined by retroviral transduction approaches. Together, these approaches should permit clinically relevant insights into the biology of residual neoplastic cells and should facilitate the development of more effective therapies targeted against this critical population of cells in patients.

Principal Investigator

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Other Participating Researchers

None

Expected Research Outcomes and Benefits

Breast cancer is typically treated by resection of the primary tumor followed by treatment with some combination of radiation, chemotherapy, and adjuvant hormonal therapy. Depending upon a spectrum of factors including tumor size and grade, lymph node status, and histological characteristics, patients will either remain disease-free following this course of treatment, or else develop recurrent breast cancer—either locally or at distant sites in the body. While the pathways leading to the initial growth of primary breast cancers have been extensively studied, far less is known about the genes and processes that govern the ability of tumor cells to survive and recur following therapy. This paucity of information is due in large part to the lack of animal models suitable for studying this critically important process.

This research strategy should help to illuminate the biology of the critical population of tumor cells that survives molecularly targeted therapies and that ultimately give rise to tumor recurrence. This project will improve our understanding of breast cancer in several important ways. First, understanding the mechanisms by which tumors recur should aid in the diagnosis and clinical management of breast cancer. Furthermore, the tumor markers and pathways identified will represent potential targets for therapeutic intervention. Drugs targeting pathways that promote survival of residual tumor cells are most likely to extend the survival of patients with dormant, residual disease.

In summary, we have developed a novel set of genetically engineered mouse models to study tumor progression and have coupled these with a unique approach to study the biology of tumor cells that are important for recurrence. This research strategy should provide important insight into the biology of breast cancer recurrence, a process that receives scant attention and remains poorly understood, but that is critically important for the survival of patients with breast cancer.

Summary of Research Completed

Residual lesions are observed in tumor bearing mammary gland wholemounts

The latency of primary tumor formation in MMTV-rtTA/TetO-neu (MTB/TAN) bitransgenic mice dosed on 2 mg/mL doxycycline is 48 days. We have reported transgene-independent tumor recurrence following doxycycline withdrawal and full regression occurring as early as 50 days and even longer than 200 days post-deinduction. After deinduction of the primary tumor and prior to spontaneous tumor recurrence, there is a period where the mammary tumor is fully regressed and the tumor is non-palpable. Wholemounts harvested at and beyond 28 days post-deinduction in tumor bearing mammary glands revealed a visible residual lesion that was not present in non-tumor bearing mammary glands (Fig. 1A). Residual lesions were distinctly visible in glands harvested as long as 112 days after full regression of the tumor.

Residual lesions are also prominent in carmine-stained tumor-bearing wholemount glands (Figs. 1B, 2A). Although the average latency of tumor induction is 48 days, glands that have been induced with 2 mg/mL doxycycline for a period as long as 56 days show no evidence of a residual lesion in mice in which there was no tumor formation (Fig. 1C). Glands induced with doxycycline showed epithelial hyperplasia at early time points up to 15 days, but after that period glands appear normal until a focal tumor is identified. This further suggests that residual lesions are the result of focal tumor formation and not from doxycycline-induced epithelial hyperplasia.

To locate and identify residual tumor cells in lesions, we developed an orthotopic model. MTB/TAN primary tumors were harvested and the isolated neu-driven epithelial tumor cells labeled with a green fluorescent histone (H2B-GFP) binding protein. Recipient mice maintained on doxycycline were injected with 2x10^5 MTB/TAN cells in the #4 mammary gland. After the formation of tumors, recipient mice were deinduced and the glands examined for evidence of residual lesions. Tumor bearing glands were positive for residual lesions (Fig. 1D, 1E), which were absent in control PBS-injected glands (Fig. 1F). This further supports the conclusion that lesions are the residue from focal tumor formation and not from doxycycline induction or injury at the injection site.

Importantly, residual lesions appeared morphologically identical to the lesions in the inducible bitransgenic model. In addition, GFP-labeled cells were located solely within residual lesions and were not observed outside of the lesion or in any other compartment of the gland (Fig. 1I). As such, this orthotopic model in which tumor cells are labeled with a nuclear H2B-GFP protein (Fig. 1G) enables the identification of tumor cells from the stromal and endogenous epithelial cells present in mammary glands bearing residual lesions (Fig. 1H).

Characterization of residual lesions

Hematoxylin and eosin (H&E) staining of residual lesions revealed an architecturally abnormal microenvironment populated by numerous cell types, including epithelial, mesenchymal, endothelial, and immune cells, surrounded by an abnormal stroma (Fig. 2B, 2D, 2E). Acinar structures were also observed on occasion (Fig. 2C), but generally the environment of the lesion

was characterized by a tortuous and chaotic extracellular milieu surrounding the many cell types present.

Examination of tissue sections containing residual lesions at higher magnification stained by H&E or Masson's Trichrome revealed the heavy deposition of collagen fibers in the microenvironment of the lesion. (Fig. 2F). Collagen was present only in the stromal compartment of MTB/TAN primary tumors, whereas recurrent tumors expressed collagen throughout the tumor, reflecting an abundant stromal component.

Further investigation by immunofluorescence with antibodies specific for collagen type I versus collagen type IV revealed the collagen present in the lesion to be type I, which was uniformly distributed throughout the lesion (Fig. 2G). Type IV collagen was found at the boundaries of the residual lesion (Fig. 2H). Strikingly, however, type IV collagen was not found in contact with H2B-GFP-positive tumor cells, which were confined within the collagen type I-positive matrix.

Macrophages are abundant in residual neoplastic lesions

Collagen has been reported to be deposited by fibroblasts as well as leukocytes. Given the low number of Fsp-1-positive fibroblasts observed in residual lesions, as well as the presence of hemosiderin in many of the lesions, we considered the possibility that macrophages were present within residual lesions.

To identify macrophages, we performed immunohistochemistry on residual neoplastic lesions using the murine anti-macrophage antibody, F4/80. We first quantified macrophages as a percentage of total cells in normal mammary adipose tissue and stroma surrounding terminal end buds, where they have been reported to reside. We then compared the percent of macrophages present in primary recurrent tumors to the percent present in the residual lesion.

In the stroma of the nulliparous mammary gland, macrophages were found to represent 7.6% of total cells present, although this percentage increased to 9.7% at terminal end ducts (Fig. 3A). Macrophages are required for terminal end bud elongation during puberty. Within MTB/TAN primary tumors, macrophages constituted as few as 1% of total cells present within epithelial regions, however in the stromal compartment of the primary tumor this number increased to 17.3% (Fig. 3B). Recurrent tumors showed no such compartmentalization and the percent of F4/80-positive cells was 19.7% throughout the entire tumor (Fig. 3C). Remarkably, F4/80-positive cells represented 22% of the total cells present in residual lesions (Fig. 3D). This unexpected finding raises the possibility that the innate immune response may play a critical role in the mechanism of tumor recurrence.

Residual lesions are well-vascularized

To investigate the status of perfusion and vascularization of residual lesions, we performed a series of immunofluorescent studies using the antibody CD31(PECAM), which is specific for endothelial cells (Fig. 4). Both primary and recurrent tumors in intact bitransgenic and orthotopic models were found to be well-vascularized with only rare areas of necrosis evident in large tumors. Tumors were positive for CD31 expression throughout the lesion, consistent with

the lack of necrosis observed in H&E stained sections of lesions. These findings suggest that angiogenesis may not be a rate-limiting step for tumor recurrence in our model system.

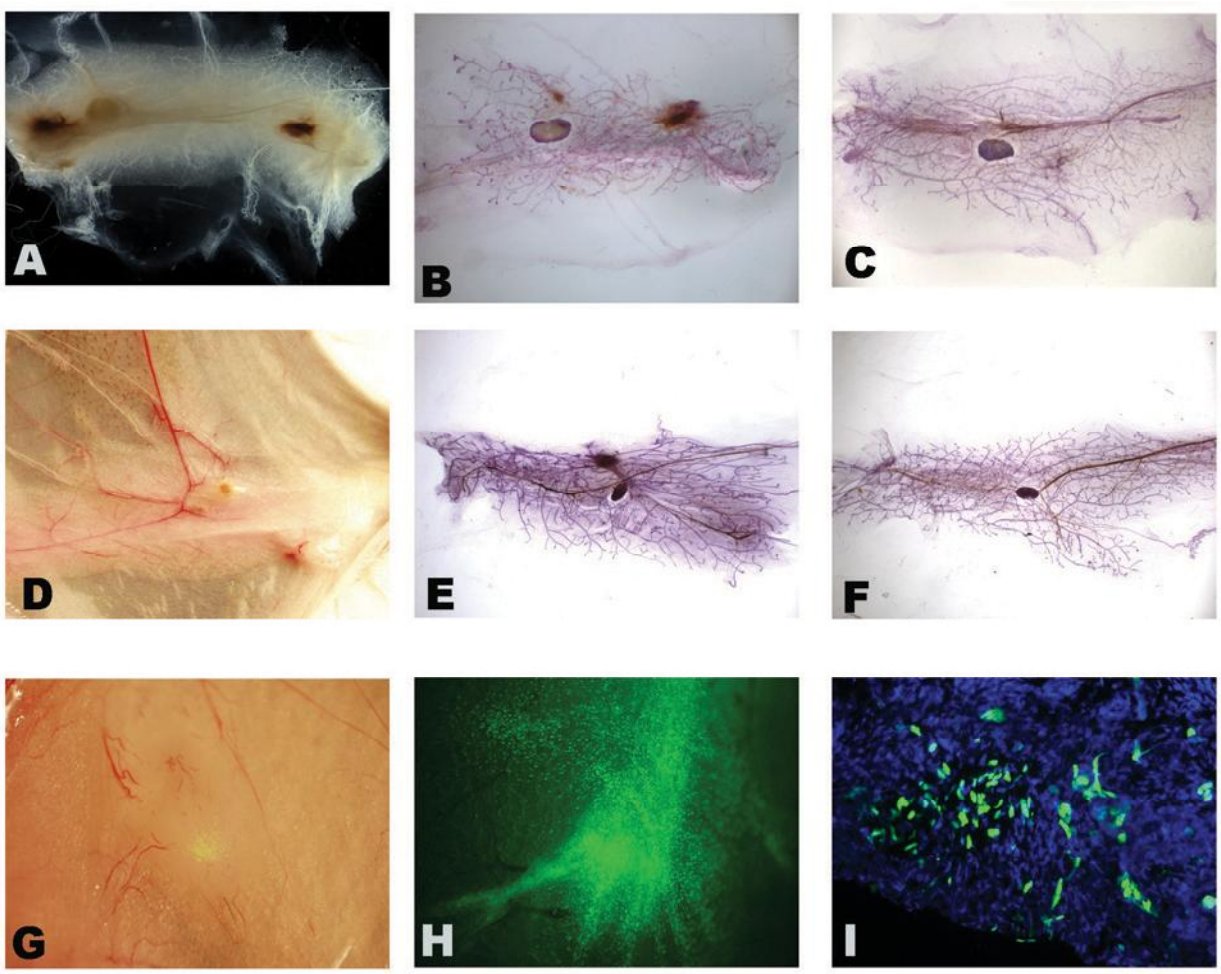

Figure 1: Identification of residual neoplastic lesions and residual tumors cells in mammary glands following HER2/neu down-regulation in mice bearing H2B-GFP-labeled primary orthotopic tumors.

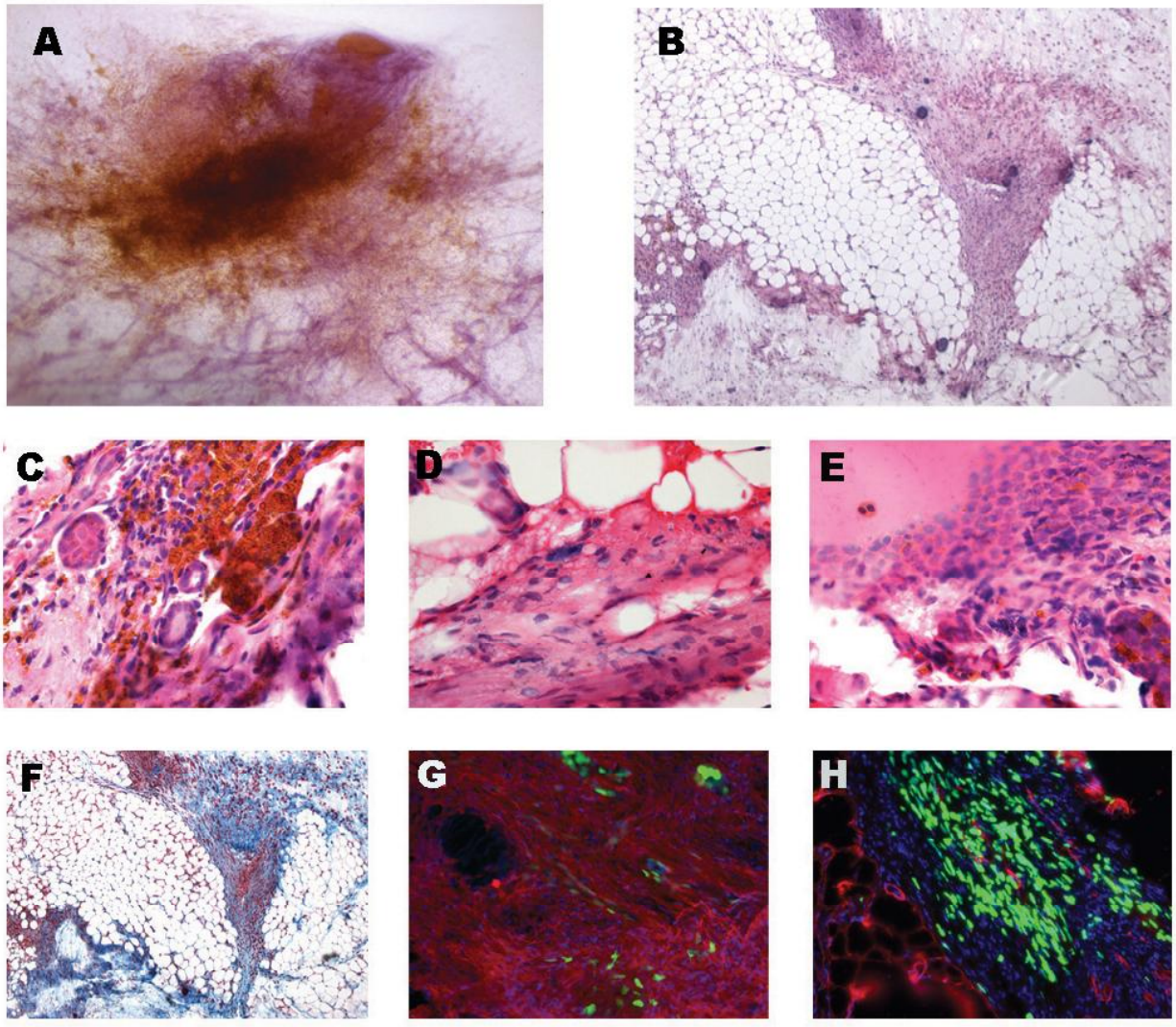

Figure 2: Histological characterization of residual neoplastic lesions.

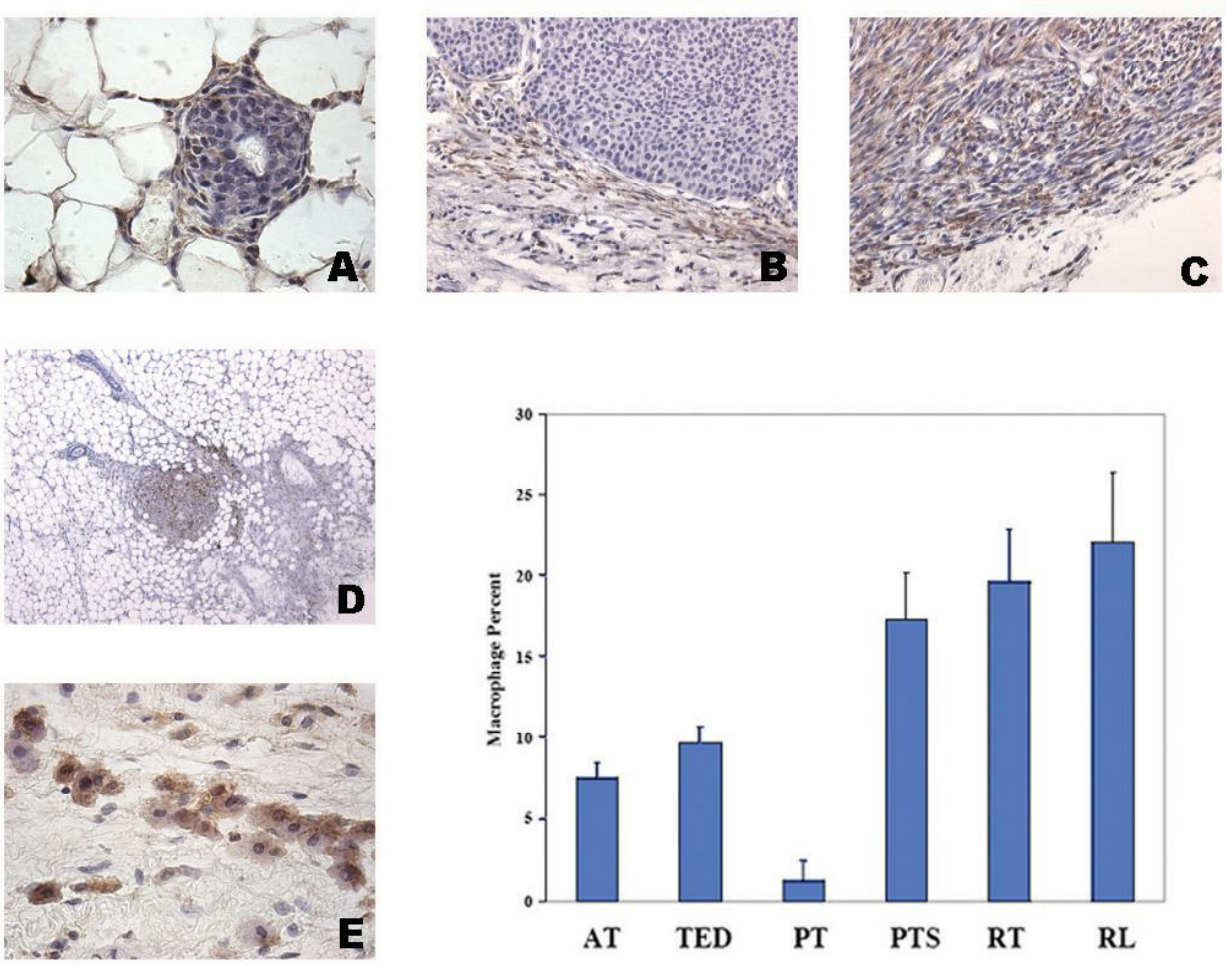

Figure 3: Immunohistochemical demonstration of abundant macrophages in residual neoplastic lesions.

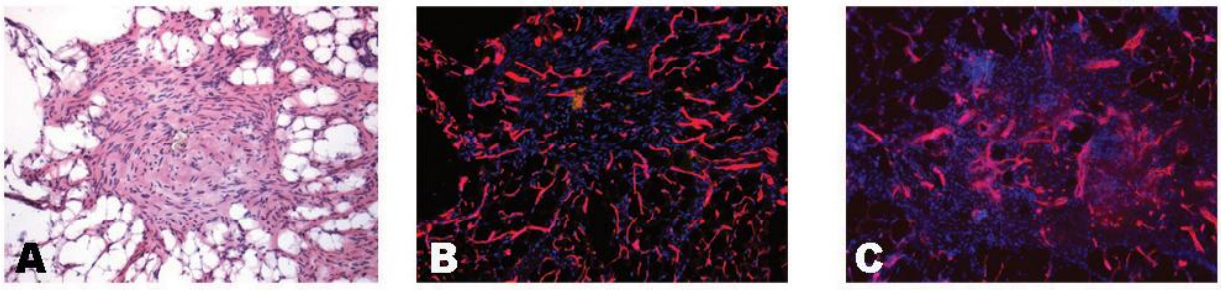

Figure 4: CD31 staining reveals that residual neoplastic lesions are well-vascularized.

Research Project 6: Project Title and Purpose

Individualizing Breast Cancer Prevention in Primary Care - The overarching objective of this project is to develop and test a multi-faceted strategy for optimizing the adoption of individualized breast cancer prevention in primary care. Specifically, we will determine whether the use of breast cancer risk reduction strategies among high risk women can be increased by providing the women targeted information and by incorporating breast cancer risk assessment and prevention into the electronic medical record of their primary care providers, and that the combination of these interventions is more effective than either intervention alone.

Anticipated Duration of Project

1/1/2009 - 12/31/2012

Project Overview

The last ten years have witnessed tremendous advances in breast cancer prevention (with FDA approval of both tamoxifen and raloxifene for primary reduction of breast cancer risk), detection (with the development of breast MRI screening and its inclusion in the American Cancer Society guidelines for screening of high-risk women), and risk assessment (with the availability of improved risk assessment models as well as the availability of genetic testing for *BRCA1* and *BRCA2* mutations). Together, these advances have the potential to substantially reduce breast cancer mortality in the US. However, this potential depends upon their effective translation into clinical practice. Unfortunately, current data suggest that this translation has been less than ideal. Recent data demonstrate that few primary care physicians have prescribed tamoxifen or raloxifene for primary prevention for breast cancer, referred patients for genetic testing, or used breast cancer risk prediction models. This project will complete the development of an integrated tool for risk assessment, pilot this tool, and begin data collection for the mammography portion of the grant.

Aim 1. To determine whether the collection, calculation and provision of individualized breast cancer risk and prevention information at the time of mammography screening increases the uptake of targeted breast cancer risk reduction strategies (chemoprevention, MRI screening, *BRCA1/2* testing) among women at risk.

Aim 2. To determine whether the inclusion of an individualized breast cancer risk assessment and prevention module in the electronic medical record (EMR) increases the uptake of targeted breast cancer risk reduction strategies among women at risk.

Aim 3. To explore whether the effects of these interventions are independent or synergistic.

Principal Investigator

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Other Participating Researchers

Jill Stopfer, MS, Jessica Tigges-Cardwell, BS - employed by University of Pennsylvania

Expected Research Outcomes and Benefits

The results of this project will substantially advance progress towards reducing breast cancer incidence and mortality in several ways. First, the project will directly impact a defined population of women, increasing the use of breast cancer risk reduction interventions in this population and thereby decreasing their subsequent breast cancer incidence and mortality. If the interventions lead to an additional 500 women at elevated risk pursuing chemoprevention, MRI screening or *BRCA1/2* testing (a relatively conservative estimate), current data suggest that between 30 and 50 fewer women in the overall population would die from breast cancer simply based upon the study itself. Second, the project will create and evaluate new tools for dissemination of individualized breast cancer prevention that can have a significant impact on the delivery of these advances in primary care and screening settings reaching across the Commonwealth of Pennsylvania. By demonstrating that relatively simple breast cancer risk assessment interventions at the point of care can increase the use of preventive interventions and that this use can be tracked through an EMR, this study has the potential to rapidly advance the dissemination of targeted breast cancer risk reduction strategies to high-risk women across the country. Third, the project will provide new insights into how to influence patient and provider behavior, including the relative impact of patient level and provider level interventions. Fourth, the project will draw attention to the importance of breast cancer risk assessment and prevention more generally, thereby encouraging investment by patients and providers in the development of new strategies for risk prediction and prevention. Although the recent advances have provided important tools for moving forward on individualized breast cancer risk reduction, it is clear that these tools remain imperfect. This project represents an early step in drawing attention to the extraordinary potential for return on investment in this new paradigm.

Summary of Research Completed

In the past year, we have used Commonwealth resources to work on alternative models of breast cancer risk stratification in order to broaden risk stratification into the general population. Although we have a well established Cancer Risk Evaluation Program at the University of Pennsylvania this serves a very limited group of patients and most women in the United States never have any type of breast cancer risk assessment. The goal of this grant is to widen access to such risk assessment specifically to women undergoing routine mammographic screening for breast cancer. In the past year Commonwealth Funds have been used to support the following:

Training of staff in risk assessment models and their use
Currently available risk assessment models require familiarity with pathology reports (atypical ductal hyperplasia, LCIS), family history (including the understanding of the impact of age and

tumor type on cancer risk assessment), and other aspects of medical history. Individuals involved in this project have been extensively trained in their use by Jill Stopfer, MS, CGC.

Education of mammography unit regarding risk assessment models and their use
The clinic based mammography reporting forms were re-worked to be more user-friendly so that patients accurately and completely fill out information. Staff has been instructed to ensure that all variables are filled out entirely.

Ongoing work with the Information Technology Group to program web-based applications for patient entry of breast cancer risk assessment.
We have purchased laptop personal computers for this purpose. Initial attempts to use an available open source program (specifically the hughesriskapp) was not successful as it did not conform to University of Pennsylvania security standards. We have investigated several other options. At the current time, members of the BMIC at the University of Pennsylvania are re-writing the program and we hope to have a web-based platform available shortly. The goal of this work is a web-based patient entry of variable for breast cancer risk assessment which is automated.

Worked with EPIC group on Electronic Medical Records (EMR) modules for breast cancer risk assessment.
The need for a breast cancer risk assessment module for the electronic medical record is critical. As such we have been educating and training the EPIC team on the variables needed for such a module.

Trained staff to work on mammographic risk assessment project : opened and enrolled on IRB approved protocol.
We were able to use the progress that we made in risk assessment and supplement this funding with a Grand Opportunity Grant from the National Institutes of Health, and wrote and opened an IRB approved protocol. This is a more expansive protocol with not only standard risk assessment, as described and funded here, but also single-nucleotide polymorphism assays. Six hundred women undergoing routine screening mammograms have been enrolled to date. Baseline information has been collected and standard risk assessment is underway. Once we have web-based and automated risk assessment available as is being funded by this grant, this will be incorporated.

Research Project 7: Project Title and Purpose

Clinical and Molecular Predictors of Responsiveness to Angiogenesis Inhibition in Advanced NSCLC - Bevacizumab is a monoclonal antibody targeting VEGF (vascular endothelial growth factor), a humoral protein that stimulates the growth of tumor vasculature (blood vessels), which, in turn, fuels the growth and spread of cancer. This agent in combination with chemotherapy, has been approved for use in "selected" patients with metastatic non-small cell lung cancer; but it has not been determined *a priori* who actually benefits from this costly, potentially toxic agent. This study will evaluate serum, tissue and radiological correlates to help determine who is likely to benefit from Bevacizumab.

Anticipated Duration of Project

1/1/2009 - 12/31/2012

Project Overview

This project will study a number of tissue and serum markers in patients with advanced, incurable non-small cell lung cancer (NSCLC) (stage IIIB and IV) who are slated to receive bevacizumab in combination with either erlotinib or with novel chemotherapy (pemetrexed and carboplatin). To date, individual investigators and cooperative groups have been unable to identify which patients are likely to benefit from this agent. There are no known molecular or clinical predictors of benefit. While bevacizumab can lead to a survival advantage in combination with standard cytotoxics, it is also costly and potentially toxic. Therefore, it's imperative to eliminate this non-specific "one size fits all" approach. The following correlatives will be assessed:

1. Serum Levels of: E-Selectin (endothelial leukocyte adhesion molecule); ICAM (intracellular adhesion molecules); VEGF (vascular endothelial growth factor), Acidic and basic FGF (fibroblast growth factor); Interleukin-8; Platelet-derived endothelial cell growth factor; transforming growth factor-α, β; Tumor necrosis factor- α; circulating endothelial cells (CECs)
2. Tissue Histology: tissue microvessel density (MVD)
3. Radiographics correlatives with real time DEC-MRI (dynamic contrast-enhanced magnetic resonance imaging), to measure tumor vascularity. FDG-PET/CT (fluorodeoxyglucose positron emission tomography integrated with computed tomography) and FLT-PET/CT (fluor-L-thymidine positron emission tomography), will also be obtained to measure tumor response to therapy and correlate these factors with clinical outcome, including toxicity, response rate (RR%); progression-free survival (PFS) and overall survival (OS).

We anticipate enrollment of 30 subjects over a 3 year period.

Principal Investigator

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Other Participating Researchers

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Expected Research Outcomes and Benefits

The following outcomes and benefits are anticipated from this project:

1. Demonstration that molecular, serum, and radiographic correlatives will predict which patients will respond to bevacizumab combinations in patients with advanced NSCLC, for whom empiric therapy has previously been the standard. Correlation of serum, tissue and radiographic correlatives with PFS and OS in advanced NSCLC patients receiving bevacizumab in combination with either erlotinib or carboplatin plus either pemetrexed or taxanes (paclitaxel or docetaxel).
2. Assess the ability of FLT-PET/CT, FDG-PET, DCE-MRI, ADC (Apparent Diffusion Coefficient) mapping to predict response to therapy as determined by conventional restaging with CT RECIST (computed tomography response evaluation criteria in solid tumors).

Summary of Research Completed

We have made headway on several fronts over the past calendar year.

We have hired a full time nurse coordinator, D. Lynn Werner, RN who is screening all newly diagnosed patients, and consenting them for biospecimen collection, including blood, urine and tissue. This cohort includes all treatement-naïve patients who will receive bevacizumab as well as other angiogenesis inhbitors. Lynn Werner will facilitate the blood and tissue collection process, endpoints 1 and 2. Biospecimen data will be correlated with vital clinical data from each patient. Dr. Anil Vachani, Lynn Werner, and I [Corey Langer] have developed a set CRFs (available on request) that will be used to collect data for this project. This CRF will ultimately be web-based and accessible to all members of the project team with continual updates, based on data entered into EPIC. These case report forms (CRFs) will track all patients screened and consented for this project and will include baseline, de-identified demographic data, including co-morbidities, as well as follow-up information regarding these patients. This form will also detail treatment administered, as well as response status, and information on progression-free survival (PFS) and overall survival (OS). Lynn Werner, has made critical changes to the CRFs to streamline data collection

We are in the process of determining a laboratory investigator at Penn who is best suited to identifying relevant polymorphisms and serum correlatives.

With regard to the imaging component [endpoint 3], there are two subsidiary, related projects, both IRB-approved:

1. We have hooked up with Dr. Sharyn Katz of nucleur medicine, who is spearheading an IRB-approved pilot study of Fluoro-labelled thymidine (FLT) as an imaging tracer for Positron Emisssion Tomography (PET/CT) for imaging unresectable lung cancer with comparison to Fluoro-deoxy glucose (FDG), the routinely used imaging tracer for PET/CT in lung cancer. To date, one of our patients has been enrolled, and her protocol coordinator, Thomas Ferrara, is actively screening additional patients. The goals of this study, which dovetail with our effort, follow:

Primary Outcome Variable(s)

a. Maximum standardized uptake value (SUV_{max}) of primary tumor uptake for FDG-PET/CT and FLT-PET/CT
b. Total uptake volume of primary tumor uptake for FDG-PET/CT and FLT-PET/CT
c. PET/CT Metabolic response

Secondary Outcome Variable(s)

a. Cinical Outcome at routine restaging after 3-4 cycles of chemotherapy
b. Time-to-progression (months)
c. 1 year survival rate (%)
d. Ki67 staining (%)

2. In addition, we are collaborating with Dr. Abass Alavi and his protocol coordinator, Tom Werner, who are spearheading a phase II, open label, non-randomized, multi-center, pilot efficacy study of [F-18] RGD K5 Positron Emission Tomography [PET] as a tool to monitor response to anti-angiogenic agenst in oncology. With the very recent IRB-approval of this study, participants are being actively screened. Funding for Dr. Alavi's protocol is from industry, and extraneous to funding for Project 7.

   In this regard, the use of DC-MRI endpoint will likely not come to fruition, unless an IRB-approved study evaluating this modality in advanced NSCLC at our institution emerges.

Two related IRB studies in bevacizumab-eligible have closed to accrual during the past calendar year. These include UPC02508: a randomized phase II of combination docetaxel and carboplatin +/- vandetanib and UPCC 03508, an investigator-initiated phase II study of carboplatin, pemetrexed, and bevacizumab led by Dr. Jamie Stevenson. One remains open: UPCC 06508 phase II study of bevacizumab and erlotinib in elderly patients.

Three related trials for advanced NSCLC patients eligible for angiogenesis inhibitors have been approved only recently during this past calendar year; they should provide an additional source of patient enrollment:

1. UPCC 20508: a randomized phase II trial of carboplatin and paclitaxel +/- ABT869
2. ECOG 2508: randomized phase II study in never smokers assigning patients to either standard treatment [paclitaxel/carboplatin +/- bevacizumab] or standard treatment plus erlotinib;
3. E3508: a randomized phase II trial of paclitaxel/carboplatin/bevacizumab +/- IMC-A12);

Research Project 8: Project Title and Purpose

Reprogramming Cells in Studies of Heart and Lung Development and Repair - Heart and lung disease are the number one and three causes of death and illness in the United States. The ability of the heart and lungs to repair themselves is very limited and poorly understood. Research into how these two organ systems respond to injury as well as try to affect repair will have a dramatic impact on both human and financial costs of these illnesses. Our project will investigate whether non-heart and lung cells can be differentiated into heart and lung cells which would provide a potential source for heart and lung repair. Such discoveries would greatly improve the outcomes of people with cardiac and lung disease.

Anticipated Duration of Project

1/1/2009 – 6/30/2011

Project Overview

The ability of the heart and lung to effect sufficient repair after chronic or acute injury is poorly understood. Recent advances in regenerative biology offer hope that a combination of stem/progenitor cell therapy and tissue engineering will promote tissue repair and regeneration through the directed activation of endogenous cardiac and pulmonary stem/progenitor cells in vivo and ex vivo or the directed reprogramming of non-cardiopulmonary cells into the cardiac myocyte and lung epithelial lineages. The long-term goal of this project seeks to harness these novel technologies and advance them to explore whether such techniques can be used to promote cardiac and pulmonary tissue regeneration. The aims of this project are to:

1) To directly reprogram adult cardiac fibroblasts to cardiac progenitor cells.
2) Generate and characterize induced pluripotential stem (iPS) cells from congenital heart disease patients to determine the molecular cause of this important category of human congenital disease.
3) Determine whether non-pulmonary cells can be directly reprogrammed into the pulmonary epithelial lineage using known and recently identified regulators of pulmonary epithelial differentiation.

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Expected Research Outcomes and Benefits

This project will define whether non-heart and non-lung cells can be transformed into useable heart and lung cells for future use in regenerative therapies. In addition, the characterization of patient derived iPS cells will greatly enhance our understanding of the causes of congenital heart disease. Although heart and lung disease are important contributors to human illness, very little is understood about their causes. These studies will reveal what genes and molecular pathways are disrupted in specific forms of congenital heart disease. Moreover, from this research we

hope to define the basic blueprint of the necessary genetic program required to generate heart and lung cells using adult cell types. The establishment of procedures to generate large numbers of heart and lung cells from readily available adult cell types such as skin cells would greatly improve the chances that heart and lung disease can be treated with these approaches.

Summary of Research Completed

Aim 1: To directly reprogram adult cardiac fibroblasts to cardiac progenitor cells.

During the current year, we have successfully isolated mouse embryonic and cardiac fibroblasts from a mouse transgenic line in which green fluorescent protein (GFP) is expressed under the control of the cardiac specific enhancer of Nkx2-5, the earliest known cardiac transcription factor. This is the same transgenic background of the embryonic stem cells used in the analysis of cardiogenesis and in determining the potential list of critical cardiac transcription factors. The need for this dynamic reporter line became apparent in analyzing previous data in which expression of constitutive LacZ from the cardiac reporter lines isolated the previous year does not imply continued expression of the genes in question. Thus it was necessary to obtain reporters in which we could readily assess the active expression of important cardiac factors. Transduction of the candidate list of cardiac transcription factors has yielded significant and sustained de-novo cardiac gene expression from non-cardiac cells, including skin fibroblasts. Upregulation of critical factors such as Nkx2-5 and Tbx5 as well as structural contractile proteins such as Myh6 can be achieved by ectopic expression of the selected list of transcription factors.

In an attempt to enhance the reprogramming efficiency and robustness we have used the histone deacetylase inhibitor valproic acid (VPA). Concomitant use of VPA enhances the induction of cardiac transcription factors from fibroblasts treated with the cardiac transcription factors. Furthermore we wanted to assess whether the induction of cardiac gene expression from skin fibroblasts is dependent on the continued ectopic expression of the candidate genes or whether we can determine the window of independence. To this end we have tried to utilize the Tet-operator/Tet-repressor (TR) system in which expression of each virally encoded gene is dependent on doxycycline. The pLOVE vector has this Tet-operator, such that without the TR expression from this vector is constitutive but when the TR is coexpressed viral expression is tet-inducible. This system has, however, been extremely difficult to optimize because achieving expression of the necessary Tet-Repressor from treated cells does not reach 100%, even when these cells are maintained under antibiotic selection. This is especially difficult when using primary cells as opposed to immortalized cell lines. In order to overcome this limitation we have inserted a gateway cassette into and are currently cloning into the FU-Tet-O vector backbone the candidate genes. This vector operates through the Tet-O system in which no expression is obtained from transgenes in the absence of both rtTA and doxycycline.

Aim 2: Generate and characterize induced pluripotential stem (iPS) cells from congenital heart disease patients to determine the molecular cause of this important category of human congenital disease.

Our work to date has now expanded beyond the generation of iPS cells from children with congenital heart disease to understanding the mechanisms of CHD formation using iPS cells.

Previously, our *in vivo* experiments using fresh source tissue from patients at the Children's Hospital of Philadelphia led to the successful production of iPS cells. We have now carried this work on considerably to completely characterize these cells and differentiate them into all developmental lineages.

1) Of the proposed library of CHD patient samples, we need just three more to finish our recruitment.
2) We have screened clones by IHC and demonstrated expression of the pluripotentiality markers NANOG, SOX2, OCT4, SSEA3, and SSEA4.
3) We have screened clones by RTqPCR and demonstrated expression of the pluripotentiality markers NANOG, SOX2, OCT4 is a quantitative manner. Importantly, we now identify that the source fibroblasts (cardiac or dermal) appear to impart an expression signature upon the reprogrammed iPS cells.
4) We have differentiated these iPS cells *in vivo* (teratoma production) and *in vitro* (embryoid body production) proving the fidelity of these iPS cells to produce multiple lineages
5) We have directly differentiated human iPS cells in cardiac myocytes and are now working to further characterize these cells by qPCR and IHC.

Our focus now is upon the directed differentiation of these cells to the cardiac lineage and the identification of assay systems that can model discrete phases of cardiac development *in vitro*.

Aim 3: Determine whether non-pulmonary cells can be directly reprogrammed into the pulmonary epithelial lineage using known and recently identified regulators of pulmonary epithelial differentiation.

Our work continues to focus on the molecular pathways that regulate lung regeneration. We continue to study the role of developmental pathways in this context with a focus on Wnt signaling and the transcription factors Gata6, Foxp1, Foxp2, and Foxp4. During the past fiscal year our work has lead to several key findings which are summarized below:

1) We have shown that Wnt signaling is required for proper lung smooth muscle development. Our current work has focused on Wnt2 and Wnt7b in lung smooth muscle development. These studies have shown that both ligands are important for smooth muscle progenitor expansion and growth post-specification in the lung. This has important implications for human pulmonary diseases such as pulmonary hypertension and asthma. One of these studies has been published in the *Journal of Clinical Investigation* in the last year and the other is in process and to be submitted by the end of the Summer.

2) We have also recently published data on the role of Foxp1/2/4 in hyperoxic lung injury and repair. These studies showed that Foxp1/2/4 interacted with the NuRD chromatin remodeling complex and this interaction repressed genes that regulated the lung epithelial hyperoxic response. The NuRD complex contains HDAC1/2 as its core histone deacetylase activity. Thus, loss of one copy of Foxp1 and one copy of HDAC2 leads to increased epithelial regeneration after hyperoxic lung injury. These studies were recently published in the Journal of Biological Chemistry.

We have identified a microRNA cluster, miR302/367, that is a target of Gata6 in the lung and acts as a regulator of lung progenitor cell behavior. miR302/367 promotes lung progenitor cell self-renewal and inhibits their differentiation. We have performed both gain and loss of function for this miRNA cluster and are currently preparing a manuscript to submit based on these findings. We have found that expression of miR302/367 in combination with Nkx2.1 and Gata6 leads to an increase in lung marker gene expression (i.e. SP-C) in fibroblast cell lines. These initial findings will be further explored over the course of the next year to determine whether a combination of miR302/367 and other transcription factors can lead to a direct reprogramming of fibroblasts to a lung epithelial lineage.

We have also shown that the miR302/367 cluster is able to directly reprogram both mouse and human fibroblasts to an induced pluripotential stem cell state. These findings are very exciting and predict that using advanced techniques of introducing miRNAs into cells without transfection procedures could lead to the establishment of iPS lines simply by introducing these miRNAs. These studies are nearing completion and a manuscript based on these findings is being prepared for publication.

Research Project 9: Project Title and Purpose

Genome-based Bio-marker Discovery and Systems Biology Engineering - Biomarkers allow for convenient diagnosis of particular states of an organism. A robust diagnostic marker is not only important for clinical treatment regimes; it also helps us understand the molecular mechanisms of diseases. For example, many mental diseases like autism are considered psychiatric diseases mainly because neural correlates have not been found. A biomarker, once established, points to possible mechanisms that ultimately might suggest potential therapeutic approaches. Thus, establishment of biomarkers for all organismal normal and abnormal processes is of great significance to both basic and clinical studies. This project aims to develop novel biomarkers using genome-scale data, through the development of novel reagents, computational modeling and analysis, and micro- and nano-engineering.

Anticipated Duration of Project

1/1/2009 – 12/31/2012

Project Overview

Biomarkers are any biological measurements that are indicative of an organism's normal and abnormal states (diseases), as well as other key characteristics of interest. Known genetic markers for disease susceptibility such as Huntington's disease, Cystic Fibrosis, and certain types of breast cancers are classic biomarkers. In recent years, genomics has led to the candidate identification of a whole suite of novel biomarkers. Genome-based biomarker discovery often utilized large-scale sequence data analysis coupled with functional genomics data to suggest that certain gene sequence or gene expression patterns are indicative of organismal states.

More recently, sensitive reporter technologies are being developed to assay in vivo cellular and physiological processes, which in turn can serve as biomarkers. An example is the development

of functional dyes that can be imaged in vivo to indicate the progression of protein inclusion bodies in neurodegenerative diseases like Alzheimer's disease. A secondary trend with respect to the discovery and the use of biomarkers is the use of systems-level engineering tools and modeling. An important function of biomarkers is their utility as predictors of future organismal states. For example, in drug development, expensive clinical trials may be considerably shortened if the right combination of biomarkers and predictive models are able to inform the researcher of future toxicity of a candidate compound. This has led to the development of systems biology modeling of entire pathways or even organismal processes to help predict the consequences of perturbation to the processes. Along with systems modeling, another trend is the use of engineering tools to develop fully automated, often miniaturized (e.g., lab-on-a-chip), experimental pipeline to utilize biomarkers for diagnostic devices.

The research group at Penn Genome Frontiers Institute has been developing interdisciplinary teams to identify biomarkers using high-throughput technologies, develop novel sensors that can be used for biomarkers, and develop new models. In this project, our goal is to bring these preliminary studies to larger scale with the following aims:

Aim 1: Develop novel biomarkers for human normal and disease states using high-throughput sequencing, cell-based screening, bio-photonics, and single-cell genomics.

Aim 2: Develop systems models of identified biomarkers and develop single-platform diagnosis devices using identified biomarkers.

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Expected Research Outcomes and Benefits

Biomarkers are efficient diagnosis and prognosis tools for organismal states, including disease states. The main idea is to be able to determine current and future states from a small set of measurements, typically at the molecular level. Typical biomarkers include genetic markers, proteins in the blood, metabolites in body fluid, as well as readouts from biosensors.

The results of this project will generate new biomarkers leveraging information and techniques

from genome sciences. Examples include new multi-genic genetic tests, novel biosensors for molecular physiology, and image-based diagnosis. These markers will enable enhanced ability to detect both normal and disease states of humans. Furthermore, this project will include system-level computational models that will help convert biomarker information into prognostic tools. Finally, micro- and nano-scale engineering projects will help develop single-platform tools for converting the discovered biomarkers into clinical tools.

Summary of Research Completed

During this reporting period, we recruited new personnel and focused on Aim 1:

Aim 1: Develop novel biomarkers for human normal and disease states using high-throughput sequencing, cell-based screening, bio-photonics, and single-cell genomics.
We carried out two projects addressing this aim. First we developed a new assay to monitor double strand breaks (DSB) of DNA in live organisms. Cellular responses to DNA damage are critically important for both suppressing and treating malignancy. The breast cancer susceptibility genes BRCA1 and BRCA2 provide two prominent examples of this interplay between DSB repair and malignancy. BRCA mutations confer high penetrance breast and ovarian cancer phenotypes and hypersensitivity to anti-cancer DNA damaging agents. The strength of the DSB repair response thus appears to be a pivotal determinant of cancer susceptibility and chemotherapeutic response. As a pilot approach we have created an active biosensor of ATM kinase activity (Figure 1). This sensor demonstrated FRET changes as indicated by a decrease in the YFP/CFP fluorescence intensity ratio specifically in response to DNA damaging agents that create DSBs. FRET changes were detected in response to 50 μM etoposide (Figure 2) and to camptothecin (not shown), both of which create DSBs by inhibiting topoisomerase enzymes in a manner such that DSBs occur in S and in G2 phases of the cell cycle. ATM kinase activity as reflected in FRET changes were detected only in the nucleus, despite the fact that the biosensor is expressed in both nuclear and cytoplasmic compartments. In addition, we have demonstrated that sensor activity is completely inhibited by the ATM specific inhibitor Ku-55933 (not shown). These initial findings set the stage for the development of transgenic zebrafish that express our ATM biosensor.

In our second project, we carried out a high-throughput cell-based screening assay using human primary macrophages to identify essential components of anti-mirobial activity against the protozoan parasite *Toxoplasma gondii*. Macrophages exhibit potent killing activity against a broad range of pathogens, and therefore represent an appealing cell type in which to carry out reverse genetic screens. Macrophages 'sense' parasites, viruses and bacteria via pathogen-associated molecular patterns, become activated by inflammatory signals and initiate transcriptional programs that typically result in cell-autonomous destruction of intracellular pathogens. The mechanisms by which this destruction is achieved, however, are not well understood. One of the most potent activators of macrophage anti-microbial function is the pro-inflammatory cytokine interferon-γ (IFN-γ). Macrophages increase transcription of >500 genes in response to stimulation with IFN-γ, yet few of these genes have been characterized in the context of infection. During the development of our cell-based screen, we also explored the ability of different activation stimuli to drive anti-microbial responses, thereby establishing robust assays to dissect complex aspects of cellular immunity.

Our assays utilized human monocytes derived from peripheral blood mononuclear cells (PBMC) that were purified by either adherence (collaboration with Ron Colman's lab, UPenn) or by counter-current elutriation (Human Immunology Core, UPenn). We expected that the latter would provide the most reliable (and largest) batches of pure monocytes. Unfortunately, inconsistent cell viability, unreliable donor supply and frequent instrument malfunction caused us to abandon this convenient source of cells. As an alternative we worked closely with Dr. Ron Colman's lab to generate mature macrophages from PBMC obtained from individual human donors. We had two main problems using these cells. First, a single donor (giving 120 ml of whole blood) yields only enough cells for a maximum of one 384-well plate. This makes a large-scale screen unfeasible. Second, these cells were effective at killing *Toxoplasma* in some experiments but not others. This batch-to-batch variation is likely due to donor-specific differences and lower cell purity. To resolve these problems we plan on using a commercial source of cells for our future experiments. We will soon evaluate cells from Biological Specialty Corporation (http://www.biospecialty.com/cell.html), a local Pennsylvania company that can supply large quantities (300 million or more) of highly pure human monocytes.

As we continued to optimize the siRNA macrophage screen we were also studying several genes identified in a high-throughput cDNA over-expression screen for factors that regulate activity of the STAT1 pathway, a critical component of the immune response to viruses, bacteria and intracellular parasites. This screen, a collaboration with Dr. Sara Cherry, was carried out at the PGFI screening core facility and it identified ~30 host transcription factors (TFs) that act as strong positive regulators of the STAT1 pathway. Over-expression of several of these TFs has a dramatic impact on myeloid differentiation and inflammatory gene expression. The conditions that we optimized for our siRNA screen will allow us to knockdown expression of these genes in primary macrophages to characterize their role in macrophage differentiation, activation in response to stimuli and killing of intracellular pathogens. Finally, we are currently drafting a manuscript to report these findings, while finishing a few key experiments to elucidate the mechanism by which these TFs regulate the STAT1 pathway.

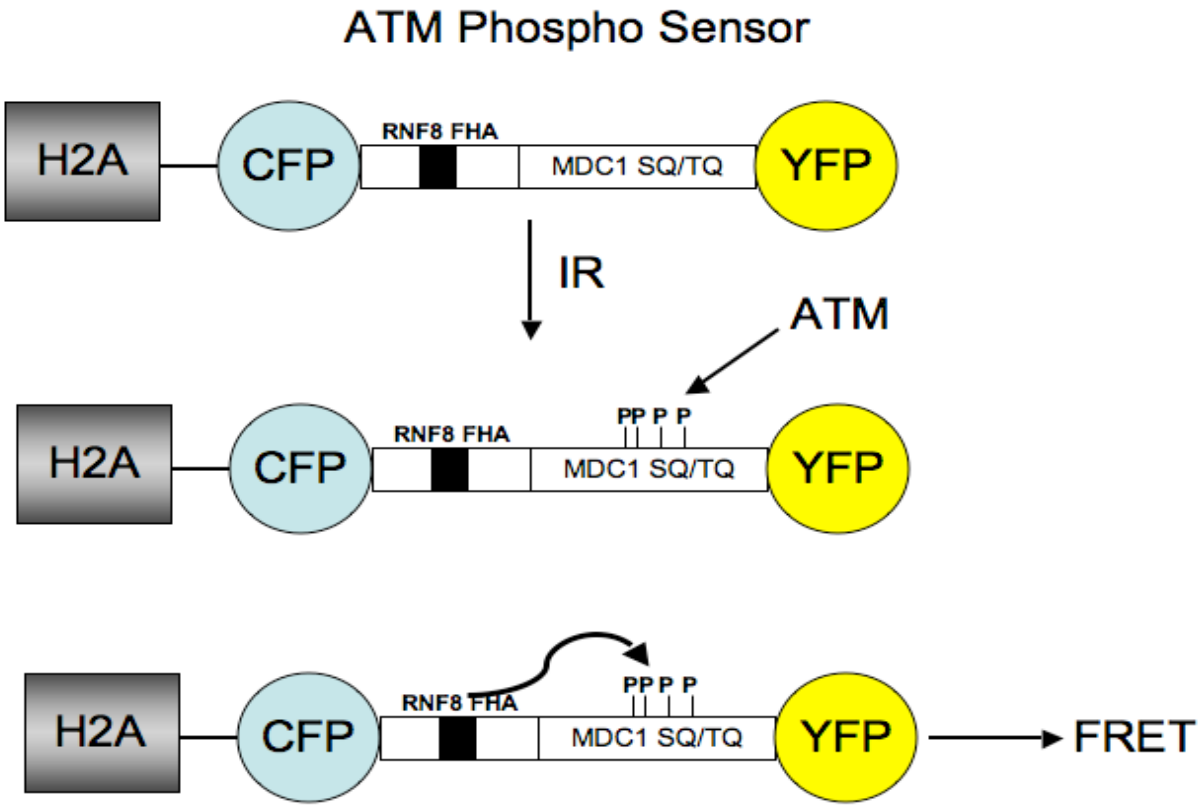

Figure 1. Development of an ATM activity sensor. The candidate ATM activity sensor is depicted as a multivalent fusion protein involving the following modules: the histone H2B protein, CFP-RNF8 FHA domain, MDC1 SQ/TQ motifs, YFP. DSB activation of ATM will initiate ATM dependent phosphorylation of MDC1 SQ/TQ motif and binding of the RNF8 FHA domain, creating proximity of CFP and YFP and FRET emission. A version of this sensor lacking histone H2A or H2B was also created (Le, Bretscher, Lampson, and Greenberg unpublished).

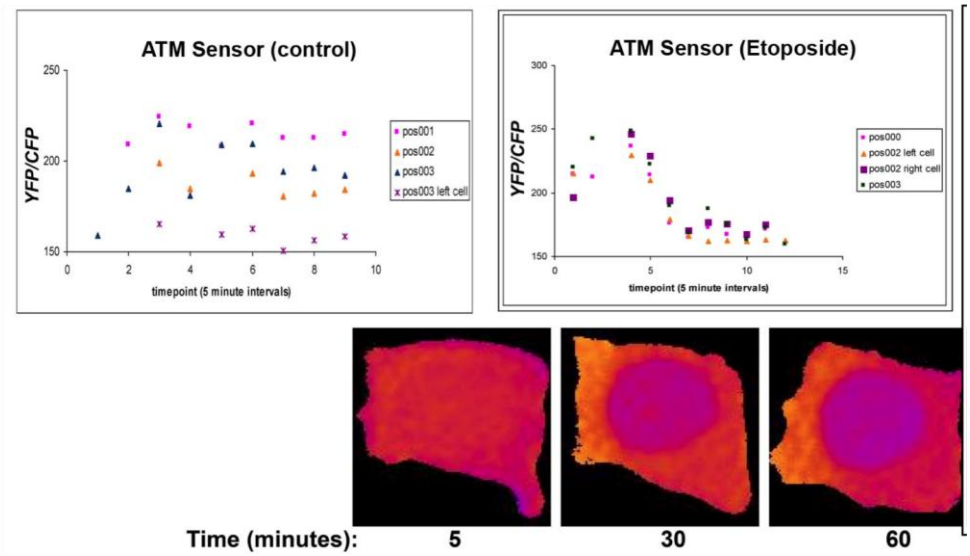

Figure 2. ATM sensor activity. The ATM sensor demonstrates nuclear FRET changes in response to 50 µM etoposide (bottom images) and to camptothecin (data not shown). FRET changes were not evident over time in the absence of etoposide (control, left) in 4 different cells. Each color in the graph represents a different cell. A decrease in YFP/CFP ratios was observed in response to etoposide. Note color changes are only evident in the nucleus after etoposide treatment (bottom images).

Research Project 10: Project Title and Purpose

Research Infrastructure: Expansion and Enhancement of Rodent Housing Space - The purpose of this research infrastructure project is to support NIH-funded research in diabetes and other diseases, as well as basic research in genetics through modification of the Clinical Research Building (CRB) animal facility. Renovation of the CRB animal facility is a critical ingredient of an institutional plan to advance Penn's mission to develop new cures through a combination of basic and clinical research. This project will allow us to achieve this goal by: (1) Renovating space to maximize cage capacity in the CRB animal facility; (2) Upgrading the ventilation system and expanding the "barrier" to reduce the spread of infectious diseases; and (3) Renovating the staff support facilities to modern standards.

Anticipated Duration of Project

1/1/2009 - 12/31/2012

Project Overview

The overall goal of this project is to improve disease prevention and control, and enable increased murine housing in the CRB vivarium. This increased capacity is essential to support critical research being conducted in the area of diabetes and other diseases, and to support basic research in genetics. In working toward this goal, we have launched a multiphase approach to renovation, which began with replacement of the tunnel washer and upgrade of the central

ventilation systems, as well as installation of a new autoclave, both of which have been completed. In the next phase of this project, we will renovate former wet lab and behavioral testing space to increase cage-holding capacity, expand the barrier to encompass the entire vivarium, and renovate staff support facilities to modern standards. Subsequent to this, we will upgrade all cage racks/cages in the vivarium to high density models. The specific aim of this requested project is to increase total CRB cage capacity from 7,063 to 15,000, including an increase in barrier capacity from 4,382 to 15,000 cages.

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None

Expected Research Outcomes and Benefits

Renovation of the CRB vivarium will have positive effects on the school's research programs by providing state-of-the-art animal care husbandry and facilities for Penn's present and future programs, including Genetics/Genomics, Metabolism (diabetes/obesity), Immunology/Transplantation and Neurosciences (behavior/neurodegenerative diseases), among others. It will increase total CRB cage capacity from 7,063 to 15,000, with barrier capacity almost tripling, from 4,382 to 15,000. It will also reduce the potential spread of infectious agents, and improve working conditions for animal care staff. Penn is committed to maintaining full accreditation with AAALAC. This facility will meet AAALAC standards, improve the quality and quantity of rodent housing, and more adequately meet the needs of NIH-funded research. Lastly, this renovation will provide essential improvements to the working environment for dedicated animal care staff.

Summary of Research Completed

Completion of design documentation occurred in June 2010. The project is going out to bid in July / August 2010. Construction in four phases will commence in November 2010 with substantial contruction completion in late 2011 – early 2012.

Figure 1 shows the new work plan and existing floor plan for the project.

Figure 1. CRB Vivarium Barrier Expansion Plan

Research Project 11: Project Title and Purpose

Development and Validation of a Tool to Assess Perceived Nutrition Environments - Obesity prevalence has reached pandemic proportions in the United States. The availability and distribution of food within a neighborhood has been related to the food intake and obesity prevalence of people living in that neighborhood. To improve our understanding of the relationship between neighborhood food environment and food intake, we need to know more about how people living in the neighborhood perceive what foods are available to them. To address this gap in the literature, the purpose of the current study is to develop a standardized measure of perceived food environment. Development of such a measure will go toward fulfilling one of the research priorities recently identified by a panel of experts for this area of work.

Anticipated Duration of Project

10/1/2009 – 10/14/2011

Project Overview

Research Objective: The study objective is to develop and validate a standardized measure of perceived nutrition environment.

Specific Research Aims:

1) To pilot-test an instrument designed to evaluate perceived nutrition environment in a convenience sample of 16 individuals.
2) To determine the psychometric properties of the instrument developed to measure perceived nutrition environment in a sample of 200 adults: 100 residing in an area of high socioeconomic status and 100 from an area of low socioeconomic status.
3) To explore whether observed nutrition environment and perceived nutrition environment are independent and additive mediators of the relationship between Self-Reported Nutrition Environment and eating behaviors.

Research Design and Methods:
This study uses a multi-phase study design that will enable the research team to develop and validate an instrument to measure perceived food environment. Phase 1 involves piloting the instrument. A draft measure will undergo internal and external review before being completed by a convenience sample of 16 adults who will also complete an audio-taped cognitive interview where they will be asked to provide feedback about the instrument content. Phase 2 of the study will involve determining the psychometric properties of the instrument. Specifically, 200 adults from geographic areas of high (Chestnut Hill and Wynnewood; N=100) and low (North and West Philadelphia; N=100) socioeconomic status will complete the instrument on two occasions (2-3 weeks apart). Study participants will also complete assessments of background characteristics (demographics, health status), self-reported community, consumer and food nutrition environments and psychosocial factors related to food intake and eating behaviors. Test-retest correlation, internal consistency and criterion validity will be ascertained from these data.

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Expected Research Outcomes and Benefits

This project is expected to yield several important outcomes that will benefit the area of obesity

prevention research and programming. The development and validation of an instrument to evaluate perceived food environment will help elucidate the relationship between observed and perceived nutrition environment and the extent to which these variables impact the relationship between food environment and eating behaviors.

Summary of Research Completed

This study is in progress, and is still in the developmental stage. This study uses a multi-phase study design to develop and validate an instrument to measure perceived food environments.

The study is currently nearing the end of Phase 1, which involves developing the initial instrument and pilot testing the instrument. A draft measure was developed based on an extensive literature search and an inventory of items.

The draft instrument was then subjected to internal and external review. There were 14 expert reviewers who provided feedback. The new draft instrument has been developed, and it has these sections: Home Food Environment; Food Shopping Questions (store food environment); Restaurant/Eating Out Questions (restaurant food environment); Your Thoughts and Habits About Food (attitudes and behaviors); and General Household Questions and Background Questions.