

# Drexel University

## Annual Progress Report: 2006 Formula Grant

### Reporting Period

July 1, 2009 – June 30, 2010

### Formula Grant Overview

The Drexel University received \$1,048,705 in formula funds for the grant award period January 1, 2007 through June 30, 2010. Accomplishments for the reporting period are described below.

### **Research Project 1: Project Title and Purpose**

*An Improved Pre-clinical Mouse Model for Scleroderma: A New Therapeutic Approach* - A better animal model for the serious and often fatal human disease scleroderma is needed. We will improve an existing mouse model to make it more like human scleroderma by breeding in an autoimmune accelerator locus (Yaa/TLR7) to enhance scleroderma signs. Yaa/TLR7 has been shown to enhance B-cell mediated disease in another mouse autoimmune model, where it caused a switch to more pathogenic antibodies that see nucleolar autoantigens present on RNA and RNA-linked entities (RNPs, RNA-polymerase). This resembles a pattern in human scleroderma patients. This modification will also provide the basis for a novel therapeutic approach to this incurable disease, using oligonucleotide inhibitors of TLR7.

### Duration of Project

1/1/2007 - 12/31/2008

### Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

### **Research Project 2: Project Title and Purpose**

*Development of an Ultrasound Contrast Agent for Detection of Ovarian Cancer* - It is estimated that there will be about 15,310 deaths from ovarian cancer in the United States during 2006. Ovarian cancer is characterized by a lack of recognizable early-stage symptoms which results in only 19% of the cases being detected at a sufficiently early stage for effective treatment. The ultrasound images generated by transvaginal sonography (TVS), currently the best method for detecting ovarian cancers, can be enhanced by the use of contrast agents which are injected intravenously before performing a scan. Under this program a new type of contrast agent, which involves the use of very small bubbles (nano-bubbles), will be developed which will improve the

sensitivity and specificity of TVS resulting in significant reduction in deaths from ovarian cancer.

### **Duration of Project**

1/1/2007 - 12/31/2008

### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

### **Research Project 3: Project Title and Purpose**

*Novel Approaches to the Treatment of Progesterone Receptor Negative Breast Cancer* - The proposed studies are designed to determine the molecular basis for the poor response of estrogen receptor positive/progesterone receptor negative (ER<sup>+</sup>/PR<sup>-</sup>) breast cancers, a tumor classification that increases with age and is refractory to the standard of treatment, selective estrogen receptor modulators (SERM), such as tamoxifen. Based on preliminary data, the transcription factor, Sp1 (a major research focus of the PI's lab for 20 years) may be a novel target for treatment of ER<sup>+</sup>/PR<sup>-</sup> breast tumors. Sp1 DNA binding is significantly decreased specifically in this class of tumors. We propose to test whether reversal of the effects of the chronic oxidative stress on Sp1 associated with aging will activate expression of PR to increase efficacy of tamoxifen treatment in ER<sup>+</sup>/PR<sup>-</sup> breast cancer.

### **Duration of Project**

1/1/2007 - 6/30/2010

### **Project Overview**

Breast cancer affects one in seven women and the incidence increases with age. Estrogen receptor/progesterone receptor (ER/PR) status of a tumor is a prognostic factor to predict the outcome of treatment and disease free survival. The majority of tumors (62%) are ER<sup>+</sup>/PR<sup>+</sup>, and the majority of these respond to selective estrogen receptor modulators (SERM), such as tamoxifen. ER<sup>+</sup>/PR<sup>-</sup> tumors comprise 13% of breast cancers with a steep age-dependent increased incidence after age 50; and have a poor prognosis as compared to ER<sup>+</sup>/PR<sup>+</sup> tumors with only 30% responding to SERM treatment. Estrogen (17β-estradiol) interacts with estrogen receptor (ER) and activates transcription of many genes through estrogen response elements (ERE) to stimulate growth and differentiation. The PR-A and -B promoters have no ERE but have binding sites for Sp1 that mediate regulation by ER. The underlying reasons for the worse prognosis of ER<sup>+</sup>/PR<sup>-</sup> tumors are not known, but one potentially significant correlation is that Sp1 DNA binding is decreased specifically in ER<sup>+</sup>/PR<sup>-</sup> tumors.

Oxidation of the zinc finger DNA binding domain of Sp1 disrupts its structure and decreases

DNA binding. The redox state of the cell changes toward an oxidized state with aging, which is thought to contribute to the increased incidence of breast and other cancers with age; Sp1 binding is decreased with age in many cancers. We have discovered that Sp1 is transiently phosphorylated by ATM in response to acute oxidative stress; phosphorylation by ATM increases Sp1 DNA binding activity in many cell types, including MCF-7 and MCF-10A. ATM is involved in maintenance of oxidative balance and regulates multiple functions (e.g., telomere maintenance, antioxidant capacity) that decrease with aging, perhaps including Sp1 phosphorylation.

This project is based on the hypothesis that chronic oxidative stress associated with aging modulates Sp1 interaction with ER (or PR) and is thereby involved in down regulation of PR and increased incidence of PR<sup>-</sup> tumors with age. To explore this hypothesis, four specific aims are proposed: 1) determine the mechanism by which chronic oxidative stress modulates Sp1 activity and its relationship to PR; 2) develop therapeutic strategies to treat ER<sup>+</sup>/PR<sup>-</sup> breast cancers based on modulating Sp1 DNA binding and interaction with ER; 3) determine the effect of oxidative stress on Sp1-dependent modulation of PR expression; and 4) determine whether oxidative stress of the stroma contributes to tumorigenesis using a co-culture system. Understanding the effect of chronic oxidative stress on Sp1 will reveal new concepts in the treatment of ER<sup>+</sup>/PR<sup>-</sup> breast cancers.

### **Principal Investigator**

Jane Azizkhan-Clifford, PhD  
Drexel University College of Medicine  
New College Building, Rm. 11102, MS 497  
Dept. Biochemistry and Molecular Biology  
245 N. 15<sup>th</sup> Street  
Philadelphia, PA 19012

### **Other Participating Researchers**

Christopher Sell, PhD, Bezhad Torabi - employed by Drexel University

### **Expected Research Outcomes and Benefits**

The majority of breast cancers in women under 50 respond to selective estrogen receptor modulators (SERM), such as tamoxifen; unfortunately, treatment failures in this group result from failure to diagnose cancers at an early stage. In patients over 50, there is increased incidence of breast cancer that does not respond to tamoxifen because of a fundamental change in the tumor that is associated with aging. The basic hypothesis of this project is that these tumors can be reverted to the class that responds to tamoxifen by reverting the tumor to the subtype more commonly seen in younger women. We propose that an alternative treatment could involve re-activating expression of a factor that is decreased with age in an effort to convert the tumor to the class that responds to standard therapy. This would involve use of agents that are used individually in treatment of cancers, but have not been used together. We propose to perform basic experiments with breast cancer cells in culture to test our hypothesis. If the cells can be converted to a state that would be expected to respond to standard therapy, such

as tamoxifen, results of these experiments would serve as the basis for a clinical trial with drugs that are in use, and as such would not require FDA approvals that could significantly delay the availability of these treatments. Within the time-frame of this grant, we expect to establish proof-of-concept.

### **Summary of Research Completed**

We made significant progress on aims 1 and 3, including developing a model of chronic oxidative stress using long-term treatment with low dose hydrogen peroxide.

The proposal was overly ambitious for the time-frame and support and aims 2 and 4 were not pursued.

In order to determine the mechanism by which chronic oxidative stress modulates Sp1 activity and its relationship to PR, shRNA was obtained to target progesterone receptor, rather than to use cell lines with varying receptor status (as originally proposed) as the different tumor cell lines are otherwise unpaired, making comparison difficult. Moreover, we reasoned that shRNA would achieve reproducible and sustained knockdown. However, at the time, the lab had no experience with using lentiviral expression vectors required for expression of shRNA. We succeeded in making lentiviruses expressing the shRNAs directed at PR and infecting MCF-7 cells. Among the 4 viruses made from the shRNA vectors obtained from Sigma, we identified one shRNA construct that gave >90% knockdown of PR in MCF-7 cells. PR knockdown decreased cell proliferation based on measurement of Ki-67 and cell counts.

To determine the effect of chronic oxidative stress on PR regulation by Sp1, initial experiments involved establishing conditions to mimic chronic oxidative stress. MCF-7 cells (ER<sup>+</sup>/PR<sup>+</sup>) were grown under conditions of alternating hypoxia and normoxia. ROS was measured using fluorescence assay to measure the oxidation of the H<sub>2</sub>DCFDA. By varying the treatment protocol (duration in each condition, level of hypoxia), conditions were established that resulted in cells with sustained increased ROS after several weeks of treatment. We spent several months doing these experiments and due to limitations of equipment results were not reproducible. Moving cells from hypoxia to normoxia and obtaining a sustained level of increased ROS, WITHOUT causing significant DNA damage presents a technical challenge.

Because of the difficulty of treating cells with alternating hypoxia and normoxia to create a condition of “chronic oxidative stress”, we sought to establish conditions for treating cells for extended times with low levels of oxidative damage. In order to create a sustained oxidative signal, this was done with *tert*-butyl hydroperoxide (TBH), which is much more stable than H<sub>2</sub>O<sub>2</sub>. To first compare the effects of TBH with H<sub>2</sub>O<sub>2</sub> (which has been extensively used in the lab to induce acute oxidative stress), cells were treated with 200 μM of each agent. The level of ROS produced was compared by measuring the oxidation of the H<sub>2</sub>DCFDA and the induction of markers of DNA damage, namely H2A.X phosphorylated on Ser 139 (γH2A.X) and Sp1 phosphorylated on Ser 101 (γSp1). The amount of damage induced after one hour and the kinetics of induction by the two agents was very similar, although removal of TBH affected the induction of damage at the later time points (Figure 1). We then sought to establish a model of chronic oxidative stress, which we did by repeated treatments with *tert*-

butyl hydroperoxide (TBH). MCF10A cells were treated with 20  $\mu$ M TBH every day for two weeks. Chronic treatment of MCF7 and MDA-MB-213 cells with 20  $\mu$ M TBH resulted in significant decrease in proliferation and marked increase in cell death, therefore a lower concentration of 10  $\mu$ M TBH was used.

To determine the biological significance of chronic oxidative stress on Sp1 binding to promoters of genes associated with estrogen- and progesterone-induced tumorigenesis and aging, we performed chromatin immunoprecipitation (ChIP) with anti-Sp1 antibodies and quantitative PCR (qPCR) of estrogen receptor alpha (ER $\alpha$ ), progesterone receptor (PgR), insulin growth factor receptor (IGF-IR), and p21<sup>Cip1</sup>. Cells were subjected to chronic oxidative stress and ChIP was performed from 10<sup>6</sup> cells using standard methods. qPCR for Sp1-binding regions in the promoters of genes of interest was performed using previously published primer sets. Chronic oxidative stress resulted in a significant decrease of Sp1 binding on the p21 promoter both in MCF7 and MDA-MB-231 cells (Figure 2a). With respect to ER $\alpha$  and PgR, chronic oxidative stress decreased Sp1 binding to the PgR receptor by 5-fold in MCF7 cells (Figures 2c and 2d), consistent with the hypothesis that PgR is a major contributor to age-related tumor progression. In contrast, chronic oxidative stress induced a significant increase of Sp1 binding in the corresponding promoters, both in MCF10A and MDA-MB-231 cells (Figures 2b and 2c). Finally, Sp1 binding to IGF-IR was 3.5-fold increased in MDA-MB-231 cells undergoing chronic oxidative stress (Figure 2e). The mechanism underlying different results obtained in different cell lines is not clear, but it appears that MCF7 is the best model in which to test the hypothesis that activation of ATM may increase Sp1 binding and PR activity in cells.

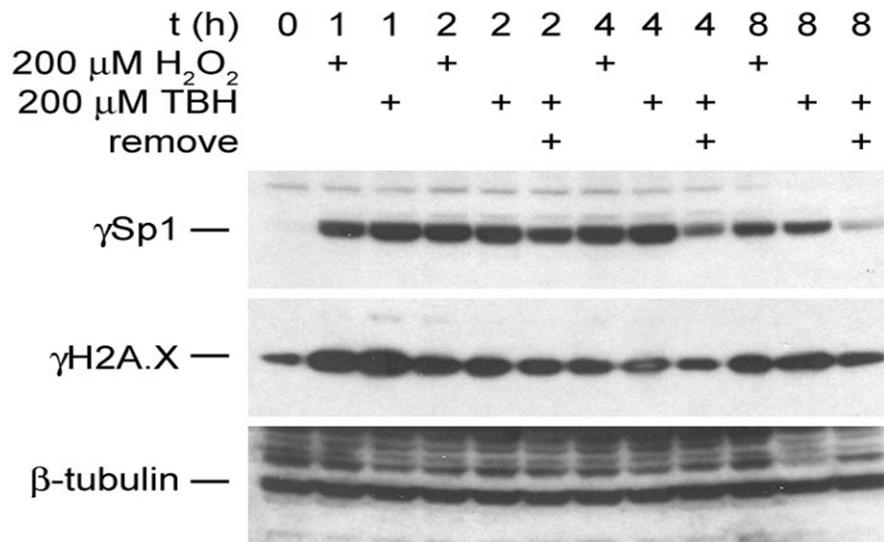
IGF-IR over-expression and p21 attenuation have been previously linked to onset of senescence. In our experiments chronic oxidative stress led to a 3.5-fold increase of Sp1 binding to the IGF-IR promoter and a 35-fold decrease to the p21 promoter (Figures 2e and 2a, respectively). MDA-MB-231 cells subjected to chronic oxidative stress exhibited enlarged nuclei (sometimes multinucleated), increased cytoplasm/nucleus ratio, and flattened appearance, consistent with a senescent phenotype (Figure 3).

Our hypothesis is that chronic ROS will decrease ATM activity. Nuclear lysates were prepared from MCF-7 cells grown in chronic oxidative stress, as well as from cells grown under normoxic conditions and acute oxidative stress. Lysates were resolved by SDS-PAGE followed by Western blot with antibody that specifically detect phosphorylated ATM pS1981 [which can be used as a measure of ATM activity], as well as antibody that detects both the phosphorylated and non-phosphorylated forms of ATM (to assess levels of expression vs. activity). Under conditions of chronic oxidative stress, the levels of phospho-ATM were found to be decreased relative to levels in cells maintained in normoxic conditions. As we have determined in other cell types, acute oxidative stress produced by treatment with higher amounts of hydrogen peroxide resulted in increased phospho-ATM. Levels of total ATM did not vary significantly under the different conditions.

To test the hypothesis that Sp1 DNA binding is decreased by chronic stress as a result of decreased ATM and accumulation of ROS, we attempted to establish the effect of decreased ATM on Sp1 binding. While siRNA was effective at decreasing ATM, we were only able to

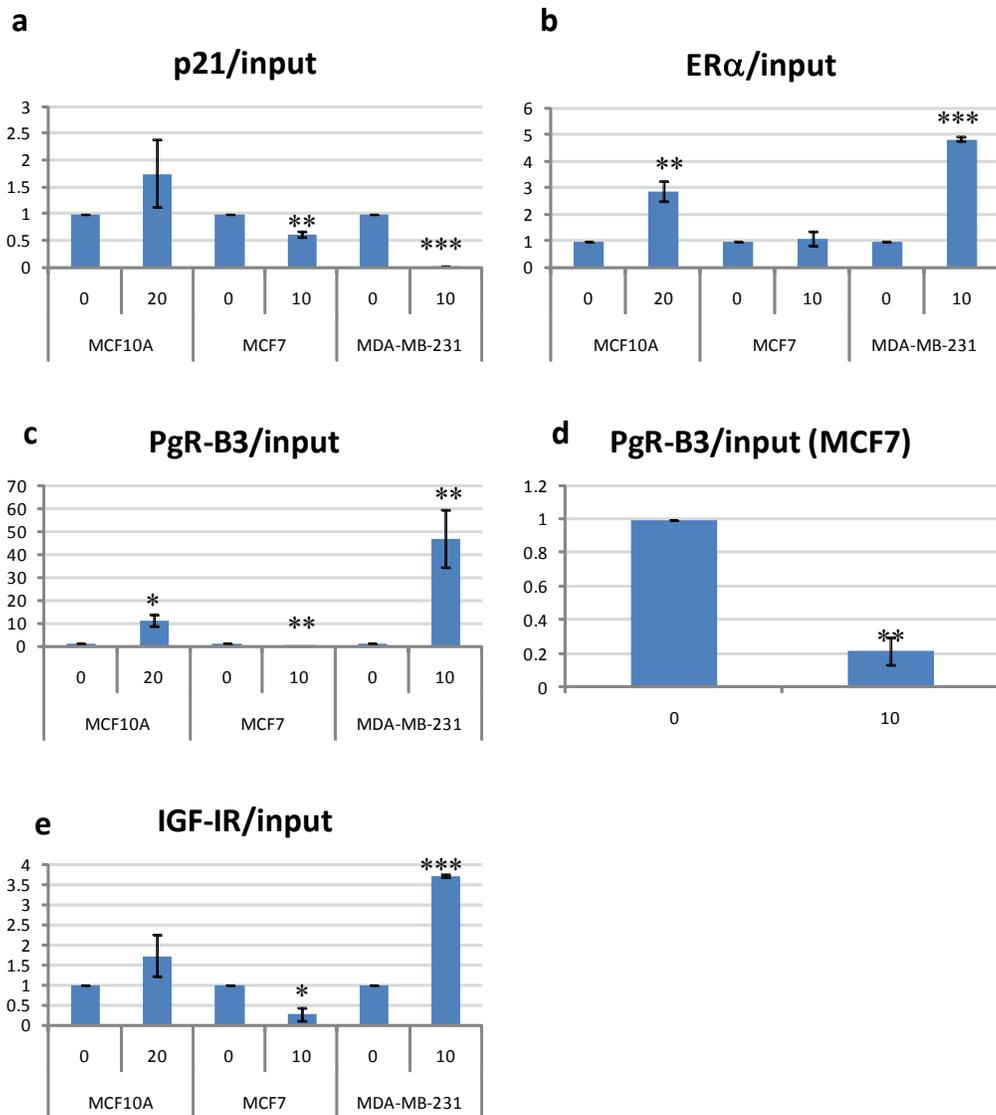
get a 50% reduction, so we purchased a set of shRNA lentiviral DNAs to target ATM. This required preparing DNA from 4 different plasmids and making 4 different lentiviruses. Virus was tested on MCF-7 cells and two of the viruses gave us ~95% knockdown. We observed that that the increase in Sp1 binding in response oxidative stress was blocked when ATM was inhibited by treatment of cells with KU55933 (10  $\mu$ M) or by ATM RNAi.

In summary, we have established a model system to reproduce conditions of chronic oxidative stress and used this system to make significant progress in establishing the relationship between Sp1 binding and chronic oxidative stress. The results obtained in the in vitro system support our initial hypothesis. We are now poised to perform some of the experiments proposed in Aim 2 to test the effect of Tamoxifen in combination with agents that increase Sp1 binding and/or activate ATM (depending on results), which should provide sufficient preliminary data for an R01 application.

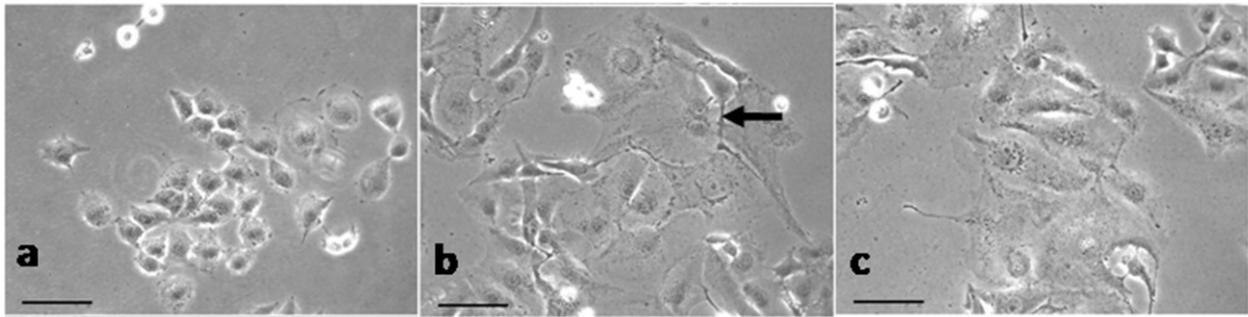


MDA-MB-231  
6/3/2010

Figure 1. MDA-MB-231 cells (ER-/PR-) were treated with 200  $\mu$ M TBH or 200  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 1, 2, 4 or 8 hours. Additionally, cells were treated with 200  $\mu$ M TBH for one hour, media were removed and replaced with fresh media without TBH, and cells were lysed at 2, 4, or 8 hours after TBH treatment (lanes indicated “remove”). Lysates were separated on SDS-PAGE. Phosphorylation of Sp1 and H2A.X was determined by immunoblotted for anti- $\gamma$ Sp1, anti- $\gamma$ H2A.X; anti- $\beta$ -tubulin was used as loading control.



**Figure 2.** MCF10A, MCF7 and MDA-MB-231 cells were subjected to chronic oxidative stress by daily treatments with 20, 10 and 10  $\mu$ M TBH, respectively, over a period of 14 days. 106 cells were used for chromatin immunoprecipitation (ChIP) with anti-Sp1 antibodies, and qPCR was performed for the specified promoter regions. Charts represent fold-change in Sp1 binding, normalized to untreated and input DNA. Error bars represent standard error of means of three replicates. Statistical significance is indicated with asterisks (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ).



**Figure 3.** Phase-contrast micrographs of MDA-MB-231 cells untreated (panel a) or treated with 10 mM TBH daily for 14 and 30 days (panels b and c, respectively). Arrow indicates binucleated cells. Scale bars 100  $\mu$ m.

#### **Research Project 4: Project Title and Purpose**

*Brain Machine Interface Control of Pelvic Robotic and Neuroprosthetic Systems* - Recently, as a result of various technical advances, direct neural control of external devices has been demonstrated. This, and advances in robotics, have enabled a series of direct neural control and neurorobotics experiments, and new types of potential therapies have been identified. Neurorobotics is the direct use of recorded neural signals to control a robot. Thus, a robot assistive orthosis or manipulator could be driven ‘by thought’. These exciting approaches are expected to enable therapies that were previously unimaginable. The advances in this area have demonstrated cortical control of arm-like robots or pointing devices. This project extends testing of ‘Brain Machine Interface’ (BMI) devices to control of the trunk and lower limb in an animal model.

#### **Duration of Project**

1/1/2007 - 12/31/2007

#### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

#### **Research Project 5: Project Title and Purpose**

*Drug Discovery for Treating Spinal Muscular Atrophy* - The goal of this project is to develop compounds that may ultimately be used in treating the deadly disease, Spinal Muscle Atrophy (SMA). SMA is caused by mutations in a critical gene called SMN1, which results in a loss of the SMN protein. The loss of SMN protein in SMA patients results in cell death in the brain,

spinal cord and muscles, leading to loss of motor function and death in early infancy (~ 2 yrs of age). The approach used in this project is to engineer novel compounds that will turn on SMN expression, and test efficacy in a transgenic mouse that mimics the human disease.

### **Duration of Project**

1/1/2007 - 12/31/2007

### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

### **Research Project 6: Project Title and Purpose**

*Roles of a Circadian Protein Timeless in Genomic Integrity and Cancer Development* - Environmental toxins or drugs that cause DNA damage lead to problems in copying DNA during cell proliferation. This causes accumulation of mutations in DNA, leading to the development of cancer. To thwart this problem, our cells monitor the accuracy of copying DNA by a mechanism referred to as a checkpoint. A protein called Timeless has been proposed to be involved in checkpoint systems. Interestingly, Timeless is also required for circadian rhythm, which controls day-night differences of our cells. However, how the Timeless protein prevents the development of cancer is unknown. Therefore, the purpose of this project is to understand the roles of Timeless in cancer development. Since a compromise in either checkpoints or circadian rhythm is known to cause cancer, results from this project should play a significant role in the treatment of cancer.

### **Duration of Project**

1/1/2007 – 6/30/2008

### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

### **Research Project 7: Project Title and Purpose**

*Targeting Protein O-GlcNAc Modifications in Breast Cancer* - In cancer a number of signaling pathways including phosphorylation/dephosphorylation cascades that add or remove phosphate residues are over-activated. Accumulating evidence suggests a less studied protein modification, known as O-GlcNAc, may globally regulate cell growth and death. The role of O-GlcNAc modifications in breast cancer phenotypes and signaling is not known. Moreover, we hypothesize that targeting enzymes which regulate O-GlcNAc modifications may be a novel therapeutic

treatment for breast cancer. Using cell biological, chemical, and proteomic approaches, this project will test the novel hypothesis that O-GlcNAc modifications regulate ERBB2 oncogenic signaling, and thus may serve as novel therapeutic targets in aggressive breast cancers.

### **Duration of Project**

1/1/2007 - 6/30/2008

### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

### **Research Project 8: Project Title and Purpose**

*Decision Making for Those with Intellectual Disabilities: A Parent's Perspective* - Mental retardation is often the diagnosis assigned to those with significant limitations in intellectual functioning and adaptive behavior. The current more acceptable terminology is intellectual and developmental disabilities (I/DD). Deinstitutionalization moved individuals with I/DD into community-based residential systems. Others, besides parents routinely make health care decisions on behalf of I/DD clients. The purpose of this project is to get information that can help professionals better understand parents' concerns and fears related to their adult children with I/DD. To date there is a lack of information for health care professionals and policy makers to truly understand parents' experiences, and the impact of the complex decisions that need to be made for advanced care planning for these disabled individuals.

### **Duration of Project**

1/1/2007 - 12/31/2007

### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

### **Research Project 9: Project Title and Purpose**

*Rapid Assay of Prostate Cancer Biomarkers in Urine for Point-of-Care Applications* - Current practice of prostate biopsy depends on digital rectal examination and prostate specific antigen levels. The statistic that only 17% (~200,000 malignant out of 1.3 million biopsies in US in 2003) of the biopsies are malignant suggests that the current practice is conservative, and improved cost-saving methods are needed. We propose to develop a method that uses the currently recognized biomarkers for prostate cancer using urine samples. Since the biomarker profile of the patient's urine can be determined in 15 minutes in the new method, the number of

benign biopsies may decline and would result in cost savings. The proposed method is possible because of recently developed high sensitive sensors in the PI's laboratory.

### **Duration of Project**

1/1/2007 - 12/31/2007

### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

### **Research Project 10: Project Title and Purpose**

*New Preterm Infant Growth Curves* - One of the primary goals for premature infants once medically stable is growth. The gold standard of growth for preterm infants is that of the fetus at the same gestational age, or intrauterine growth. The reference growth curves of intrauterine growth that are used in the clinical and research settings to compare an infant's size to the gold standard are either based on old data, heterogeneous, non-U.S. data, and/or lack a measure of body proportionality. The goal of this project is to develop a new set of growth curves for the assessment of growth status of premature infants in the clinical and research settings based on a large, U.S. population-based set of data.

### **Duration of Project**

1/1/2007 – 12/31/2008

### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

### **Research Project 11: Project Title and Purpose**

*Neural Mechanisms of the Contextual Interference Effect: An fNIRs and EEG Study* - The overall goal of this study is to gain insight into the neural mechanisms of learning multiple tasks. By examination of cognitive and behavioral output during the performance and learning of several computer maze tasks, and through a detailed examination of the neural activity obtained from functional near-infrared spectroscopy (fNIRs) and electroencephalography (EEG), it may be possible to gain insight into the impact of the amount of practice and the organization of practice on learning fine motor skills. This insight may provide direction as to how to better develop instructional and rehabilitation protocols in addition to clinical interventions to facilitate the recovery of function, relearning and transfer of cognitive and fine motor skills based upon neural responses to physical practice.

### **Duration of Project**

1/1/2007 - 12/31/2008

### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

### **Research Project 12: Project Title and Purpose**

*Identifying Pyrazolourea Target in Malaria Parasites* - In our investigations of a series of compounds that were identified through *in silico* screening based on myosin motor components of malaria parasites, we have found two compounds with low nanomolar inhibition activity against *Plasmodium falciparum* (*P. falciparum*). Interestingly, further biochemical, genetic and biophysical work suggests that the target of these lead compounds is likely to be other than the myosin light chain proteins. Our purpose is to identify the target of these compounds with the hope that this could lead to optimization of the lead compounds.

### **Duration of Project**

3/24/2009 - 6/30/2009

### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.