

# **Pennsylvania State University**

## **Annual Progress Report: 2006 Formula Grant**

### **Reporting Period**

July 1, 2010 – December 31, 2010

### **Formula Grant Overview**

The Pennsylvania State University received \$7,201,999 in formula funds for the grant award period January 1, 2007 through December 31, 2010. Accomplishments for the reporting period are described below.

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

*Regulation of Gene Expression during Inflammation* - Inflammation must be tightly controlled to prevent damage to host tissues; however, there is little known about the signals and molecular events that prevent excessive inflammation and tissue destruction. Furthermore, how these signals lead to the suppression of genes that mediate inflammatory responses and the activation of genes that promote wound repair has not been investigated. We propose to look at signals and mechanisms that regulate the macrophage receptor tyrosine kinase RON, which has been shown to temper the response of macrophages *in vitro* and *in vivo*. We will employ animal models and molecular approaches to gain insights into how macrophages balance activities associated with inflammation versus wound repair during a normal immune response.

### **Duration of Project**

4/1/2007 – 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>.

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

*Meprins – Metalloproteinases of the Kidney and Intestine* - Urinary tract infections are one of the most common bacterial infections in humans, and around 50% of women are affected at some point in their lifetimes. There are multiple factors involved in recurrent infections; however, there is a need to understand the mechanisms involved to develop more effective therapies and prevent renal scarring associated with infection. We have found that meprins are expressed by leukocytes and that meprins can cleave cytokines as substrates *in vitro* to an active form or to a non-active, degraded form. Use of unique meprin knock-out mice as models for inflammatory

diseases such as urinary tract infection and ischemia/reperfusion (I/R) induced acute renal failure show that: 1) mice lacking the meprin  $\alpha$  subunit are more susceptible to kidney and bladder infections and 2) mice that lacked the meprin  $\beta$  subunit were more protected against I/R induced kidney injury. The cytokine cleavage data, the presence of meprin expression on leukocytes, and the data accumulated from the mouse models of disease indicate a role for meprins in modulating inflammatory responses.

### **Duration of Project**

1/1/2009 – 6/30/2010

### **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**

*Glucocorticoid/Stress Effects on Dendritic Cell Function* - There is substantial evidence for psychological stress-induced, neuroendocrine-mediated modulation of the immune function. The long-range goal of this project is to define the mechanisms by which stress and its associated increase in corticosterone affect cytotoxic T lymphocyte (CTL) responses. An efficient and robust CTL lymphocyte response is necessary for the successful defense against many diseases that are immunologically resisted, in particular, virus infections and some tumors. The efficiency and robustness of this response is dependent upon the efficient functioning of dendritic cells. In this project, those components of crucial dendritic cell functions that are affected by stress and glucocorticoids and the resulting impact on the generation of CTL responses will be identified.

### **Duration of Project**

9/1/2007 – 8/31/2008

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

*Cellular Machinery in Cytomegalovirus-mediated MHC Class I HC Degradation* - Human cytomegalovirus is a beta-herpesvirus that infects approximately 70% of our population. The genome of this virus encodes specific genes that, when expressed as proteins, interfere with the detection and destruction of infected cells by the human immune systems. In one mechanism of

immune evasion, the virus expresses a protein known as US11 that causes the degradation of MHC class I heavy chain proteins, leading to the absence of these proteins in infected cells. MHC class I heavy chain proteins at the surface of infected cells normally signal to immune cells the presence of viral infection. Thus, the absence of this signaling protein enables infected cells to escape detection. The purpose of this research is to identify the human machinery co-opted by the virus US11 protein for this evasion, with the long-term goal of designing therapeutic interventions.

### **Duration of Project**

4/1/2007 – 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 5: Project Title and Purpose**

*Cytosol-Vesicle-Vacuole Protein Degradation Pathway* - Protein degradation is critical for cell cycle division, cell growth control, transcriptional regulation and metabolic control. The gluconeogenic enzyme fructose-1,6-bisphosphatase (FBPase) is degraded when yeast cells are shifted from poor carbon sources to fresh glucose, and this degradation prevents energy futile cycles that are harmful to cells. FBPase can be degraded either in the proteasome or in the vacuole depending on the duration of starvation. Our long-term goal is to understand the mechanisms underlying the vacuolar dependent pathway of FBPase degradation. The objective of this application is to understand why FBPase switches its degradation from the proteasome to the vacuole. We will test the hypothesis that the switch is controlled by multiple protein complexes that can be activated or inactivated depending upon the duration of starvation.

### **Duration of Project**

1/1/2007 - 12/31/2010

### **Project Overview**

Autophagy is a process whereby lysosomes degrade cytosolic proteins and organelles when cells are starved of nutrients. Defects or changes in autophagy have been linked to cancer development, neuromuscular dystrophies and aging. Multiple forms of autophagy exist, and a unique autophagy pathway has been identified in our lab. The gluconeogenic enzyme fructose-1,6-bisphosphatase (FBPase) is degraded when yeast cells are shifted from poor carbon sources to fresh glucose, and this degradation prevents energy futile cycles that are harmful to cells. FBPase can be degraded either in the proteasome or in the vacuole depending on the duration of starvation. For the vacuolar pathway, FBPase is first targeted to Vid vesicles and then to the vacuole. A number of *VID* genes function in both degradation pathways and they are

evolutionarily conserved. The Vid pathway is utilized for multiple cargo proteins including isocitrate lysase, phosphoenopyruvate carboxykinase and malate dehydrogenase. The objective of this project is to understand the molecular mechanisms mediating the switch of degradation from the proteasome to the vacuolar pathway. *Our central hypothesis states that the site of FBPase degradation is controlled by multiple protein complexes that are activated or inactivated depending upon the duration of starvation.* We plan to test this hypothesis by pursuing the following aims. 1. We will study why the Vid vesicle trafficking pathway is inactive in short termed starved cells. Is this because of an inactive cAMP signaling pathway, the absence of Vid vesicles, or incompetent Vid vesicles? 2. FBPase physically interacts with components of the Tor1 complex (TORC1). We will study how Tor1 regulates the vacuolar pathway. 3. Vid28p and Vid30p form a stable complex. We will study how this complex regulates both degradation pathways. The completion of the proposed experiments will enhance our understanding regarding how these two major proteolytic pathways are regulated. This may provide therapeutic advantages to eliminate abnormal proteins that accumulate in Parkinson's disease, Huntington's disease, or other pathological conditions. The proposed aims are expected to establish the roles of signaling molecules and the Vid28p/Vid30p complex in controlling the switch from the proteasome to the vacuolar pathway. Such results will have important implications, since knowing how the switch works may ultimately lead to the development of therapeutic strategies to eliminate abnormal proteins that accumulate under pathological conditions

### **Principal Investigator**

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

Our experiments will allow us to determine if the inability of 1d cells to degrade FBPase in the vacuole is due to the fact that Vid vesicles are not formed or if they are import incompetent. We expect that there is an overall decrease in proteasome function and an increase in vacuolar functions with prolonged starvation. We will also learn whether differential ubiquitination of FBPase or differential regulation of ubiquitin conjugating enzymes in 1d versus 3d cells accounts for the switch. Our experiments should reveal whether the cAMP pathway is inactive in cells starved for 1d. For Aim 2, we will test the working hypothesis that TORC1 regulates one or multiple steps of the early parts of the FBPase pathway. Although FBPase binds to both Tor1p and Tco89p, our results suggest that Tor1p is inhibitory, while Tco89p is stimulatory, and they may exert their functions at different steps of the pathway. We expect that Tco89p positively

regulates early steps of the vacuolar FBPase degradation pathway, at the step of FBPase inactivation, phosphorylation or import into Vid vesicles. The experiments should also reveal whether TORC1 plays a role in the proteasome pathway. For Aim 3, we will study the functions of Vid28 and Vid30p in vacuolar dependent degradation of FBPase. Both proteins contain protein-protein interaction domains. We have found that the ARM4 domain is required for the interaction of Vid28p with Vid30p during vacuolar degradation. We expect that the LisH domain of Vid30p is required for vacuole degradation, but not proteasome degradation. We expect that other sequences of Vid28p or Vid30p are also required, either for the proteasome pathway, for the vacuole pathway, or for both. If we understand the molecular mechanisms regulating the switch of protein degradation from the proteasome to the vacuolar pathway, we can utilize the information to activate the vacuole pathway when proteasomal function is compromised. Alternatively, we can inactivate the vacuole pathway when excessive vacuolar degradation produces harmful effects to cells.

### Summary of Research Completed

As mentioned in the previous annual report, we have received additional grant funds to investigate the role of the major PI3K Vps34p in the Vid pathway. We have gained a better understanding of the functions of Vps34p in the Vid pathway.

#### 1. Vps34p and Vps15 are involved in FBPase degradation in the vacuole.

We have shown previously that multiple gluconeogenic enzymes such as FBPase, isocitrate lyase (Icl1p), phosphoenolpyruvate carboxykinase (Pck1p) and malate dehydrogenase (MDH2) are induced when cells are starved of glucose. When glucose is added to cells that are starved for one day, these enzymes are degraded in the proteasome. Interestingly, when glucose is added to cells that are starved for 3d, these proteins are degraded in the vacuole. To study whether or not Vps34 is involved in the proteasomal or the vacuolar pathways, we examined FBPase degradation in 1d starved versus 3d starved cells lacking the *VPS34* gene (Fig. 1). FBPase was degraded normally in 1d starved cells lacking this gene. However, FBPase degradation was defective in 3d starved  $\Delta vps34$  mutant. Therefore, *VPS34* appears to be required for the vacuole dependent degradation of FBPase. Vps15p is a protein kinase that activates Vps34p. When glucose was added to 1d starved cells lacking *VPS15*, FBPase was degraded. By contrast, FBPase degradation was defective in 3d starved mutant lacking this gene. Thus, both *VPS15* and *VPS34* are required for FBPase degradation in the vacuole dependent degradation pathway.

#### 2. FBPase inactivation is impaired in cells lacking *VPS15*.

During glucose starvation, FBPase is induced and exhibits high activity. However, following the addition of glucose, FBPase is inactivated. FBPase inactivation is mediated by the cAMP pathway, as mutants lacking all three subunits of the protein kinase A failed to inactivate FBPase. Furthermore, when *TOR1* was overexpressed in wild type cells, FBPase failed to be inactivated, suggesting that excessive Tor1p is inhibitory for FBPase inactivation. We next determined whether or not *VPS34* and *VPS15* had similar effects on FBPase inactivation. Wild type and mutant cells lacking either *VPS34* or *VPS15* were glucose starved for 3d and shifted to glucose for 60 min. FBPase activity was examined (Fig. 2). For these experiments, wild type

cells were used as positive controls, whereas the FBPAse deletion strain was used as a negative control. In wild type cells, FBPAse activity was high prior to glucose addition. However, most of the FBPAse was inactivated (17.7% remaining) following the addition of glucose to cells for 60 min. In the  $\Delta vps34$  strain, FBPAse activity was also high during glucose starvation, and its activity decreased (26.1% remaining) in response to glucose. When FBPAse activity was determined in the  $\Delta vps15$  strain, FBPAse was active before glucose addition and the activity decreased to 51.3% of the original level after glucose addition. Therefore, *VPS34* and *VPS15* do not have the same effects on FBPAse inactivation. Cells lacking *VPS15* appear to be more defective in FBPAse inactivation than cells lacking *VPS34*.

### 3. FBPAse association with Vid vesicles requires Vps34p and Vps15p.

For the Vid dependent pathway, FBPAse was in abundant intracellular structures in vivo, as visualized by immunoEM. Three FBPAse containing structures have been purified. Vid vesicles are small round shaped structures. These vesicles are 30-50 nm in diameter and appear to be surrounded by smooth layers of protein coats. Vid24p and Sec28p are peripheral proteins on Vid vesicles. The second and the third FBPAse containing structures have recently been isolated using various combinations of sizing chromatography, sucrose density gradients and differential centrifugation. The second FBPAse-containing structures are called small Vid/endosomes, because they contained FBPAse, Vid vesicle protein Vid24p and the endosomal marker Pep12p. The third FBPAse-containing structures are called large Vid/endosomes and they also contained FBPAse, Vid24p and Pep12p. Using differential centrifugation, small Vid vesicles are enriched in the 200,000 g pellet (P200) fraction, whereas Vid/endosomes are enriched in the 100,000 g pellet (P100) fraction.

We next determined whether or not *VPS34* or *VPS15* have a role in the association of FBPAse with Vid vesicles using differential centrifugation (Fig. 3). In wild type cells, FBPAse was distributed in both Vid vesicle enriched (P200) and the cytosol enriched (S200) fractions. In cells lacking *VPS34*, most of the FBPAse was in the S200 fraction and minimal amount was in the Vid vesicle fraction. Likewise, in cells lacking *VPS15*, most of FBPAse was in the S200 fraction, and levels of FBPAse in the Vid vesicle enriched fraction were reduced. Since FBPAse levels in the Vid vesicle fraction decreased in the absence of *VPS34* and *VPS15*, these genes are likely to be involved in FBPAse association with Vid vesicles.

### 4. FBPAse association with Vid/endosomes/actin patches is prolonged in cells lacking *VPS15* or *VPS34*.

Reduced levels of FBPAse in the Vid vesicle fraction in these mutants could result from defects in Vid vesicle formation. Since Vid vesicles exist as free forms (30-50 nm in diameter) or clustered forms as Vid/endosomes (2-3 microns in diameter), we next determined whether or not FBPAse was associated with the clustered forms of Vid/endosomes in the absence of the *VPS34* or *VPS15* genes. Vid/endosomes can be visualized by fluorescence microscopy and they often associate with actin patches. As such, we first studied whether or not FBPAse associates with actin patches in cells lacking *VPS34* or *VPS15*. Cells were transformed to express FBPAse-GFP, glucose starved for 3d and shifted to glucose for indicated time points. Cells were permeabilized and actin was visualized by rhodamine conjugated phalloidin. In cells lacking *VPS34* or *VPS15*,

FBPase association with Vid/endosomes/actin patches was prolonged. Thus, it appears that *VPS34* and *VPS15* have a role in the dissociation of Vid/endosomes from actin patches.

5. Vps34p and Vps15p are localized to Vid/endosomes/actin patches.

We next determined the distribution of Vps34p in wild type cells that expressed Vps34p-V5-His6 using differential centrifugation (Fig. 4). Cells were glucose starved for 3d and shifted to glucose for 0 and 60 min. Cells were homogenized and cell lysates were subjected to differential centrifugation at 1,000 g, 13,000 g, 100,000 g and 200,000 g. Using this protocol, the nuclei and the plasma membrane are enriched in the 1,000 g pellet (P1) fraction, whereas the vacuole is enriched in the 13,000 g pellet (P13) fraction. Vid/endosomes and Vid vesicles are enriched in the 100,000 g pellet (P100) and 200,000 g pellet (P200), respectively. In wild type cells, most of the Vps34p was in the P100 fraction and lower levels were found in the P1 and P13 fractions. Note that very little Vps34p was in the P200 fraction that contained Vid vesicles, suggesting that most of the Vps34p is associated with Vid/endosomes, whereas very little of this protein is present on free Vid vesicles.

Since Vps34p is enriched in the P100 fraction containing Vid/endosomes, actin should also be present in this fraction. To investigate this, wild type cells expressing actin binding protein Abp1p that was tagged with mCherry were glucose starved for 3d and subjected to differential centrifugation. Indeed, a percentage of Abp1p-cherry was detected in the P100 fraction. Furthermore, most of the actin was also present in the P100 fraction. These results are consistent with the notion that Vps34p localizes to Vid/endosomes/actin patches.

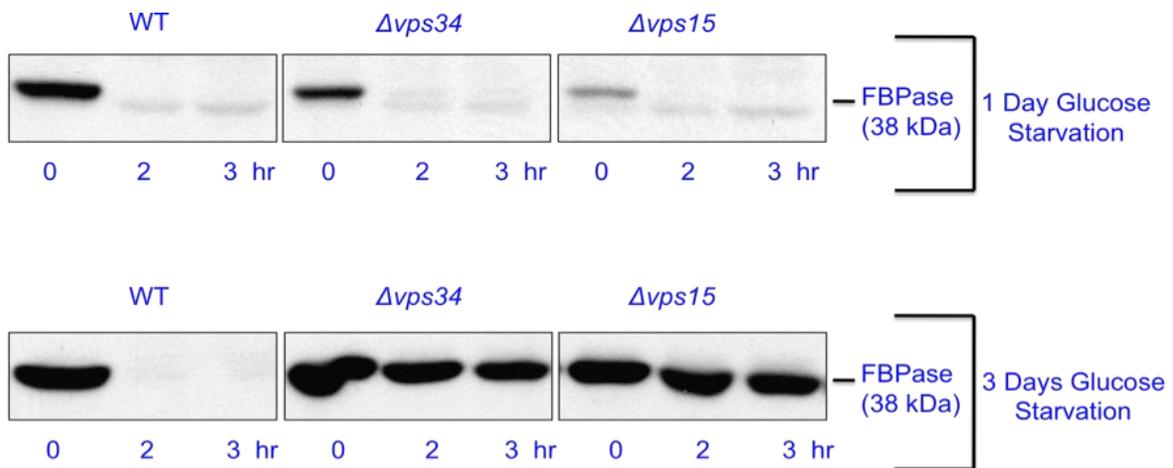
To confirm that Vps34p was distributed to actin patches, Vps34p-GFP was expressed in wild type cells and the localization of Vps34p with actin patches was determined (Fig. 5A). Vps34p-GFP co-localized with rhodamine conjugated phalloidin in 3d starved cells. Likewise, Vps15p-GFP also co-localized with actin patches in 3d starved wild type cells expressing Vps15p-GFP (Fig. 5B). Therefore, Both Vps34p and Vps15p are distributed in actin patches in 3d starved wild type cells.

Actin is present on the plasma membrane and involved in endocytosis. Actin is also detected on the vacuole membrane and involved in homotypic vacuole fusion. For endocytosis, actin polymerization occurs in a stepwise manner. Sla1p is recruited to the sites of actin assembly during early steps of the process. If actin polymerization is required for Vps34p to be recruited to actin patches, the *Δsla1* mutant that impairs actin polymerization should affect Vps34p distribution to these sites. To test this, the *Δsla1* mutant was transformed to express Vps34p-GFP. Cells were glucose starved for 3d and shifted to glucose for the indicated time points (Fig. 5C). As expected, Vps34p association with actin patches was reduced in the *Δsla1* mutant.

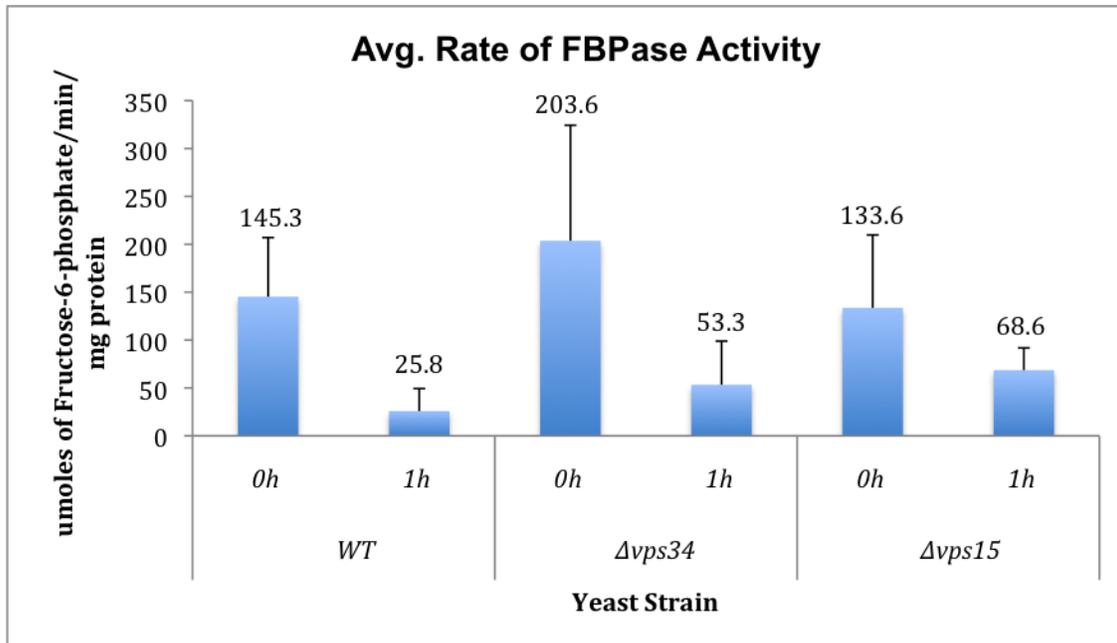
Actin polymerization also requires the Arp2/3 complex that acts at a later step of actin polymerization. The Arp2/3 complex consists of multiple protein components including the non-essential protein Arc18p. We next determined whether or not Arc18p has a role in Vps34p distribution to actin patches. Cells lacking *ARC18* were transformed to express Vps34p-GFP and starved of glucose for 3d. Cells were then shifted to glucose and examined for actin and GFP distribution (Fig. 5D). In this strain, Vps34p-GFP was diffuse. However, actin patches were still

observed in cells lacking *SLA1* or *ARC18* as shown by punctate structures containing rhodamine conjugated phalloidin (Figs. 5C and 5D). One explanation is that actin patches on the plasma membrane are affected, whereas the patches on the vacuole membrane are not affected by these mutations.

In summary, our results indicate that Vps34p and Vps15p play important roles in the vacuolar dependent degradation of FBPase. Both Vps34p and Vps15p are associated with Vid/endosomes/actin patches. This association appears to require actin assembly, as cells lacking *SLA1* or *ARC18* that are required for early and late steps of actin assembly impaired Vps34p distribution to actin patches. In the absence of Vps15p or Vps34p, FBPase failed to dissociate from actin patches. Moreover, FBPase levels in the Vid vesicle enriched fraction were reduced. We hypothesize that dissociation of Vid/endosomes from actin patches is important to generate free Vid vesicles. This is consistent with the idea that Vid vesicle biogenesis is linked to either anterograde transport to the vacuole or retrograde transport from the vacuole. As such, when dissociation of Vid/endosomes from actin patches is impaired, Vid vesicle formation is affected.



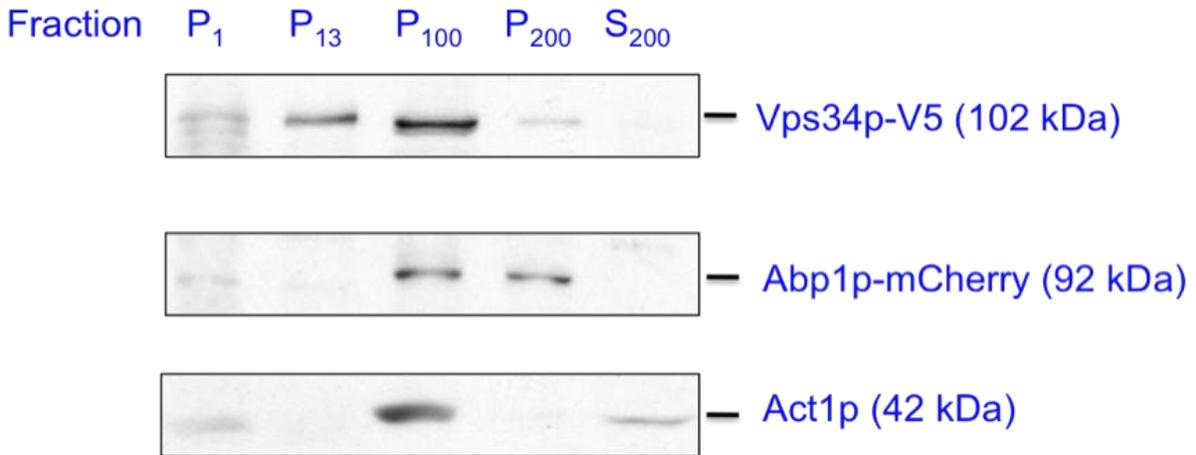
**Figure 1.** *VPS34* and *VPS15* are required for FBPase degradation in the vacuole. Wild type, the  $\Delta vps34$  and  $\Delta vps15$  strains were glucose starved for 1d and 3d. Glucose was added to cells for 0, 2 and 3 hours. FBPase degradation was examined.



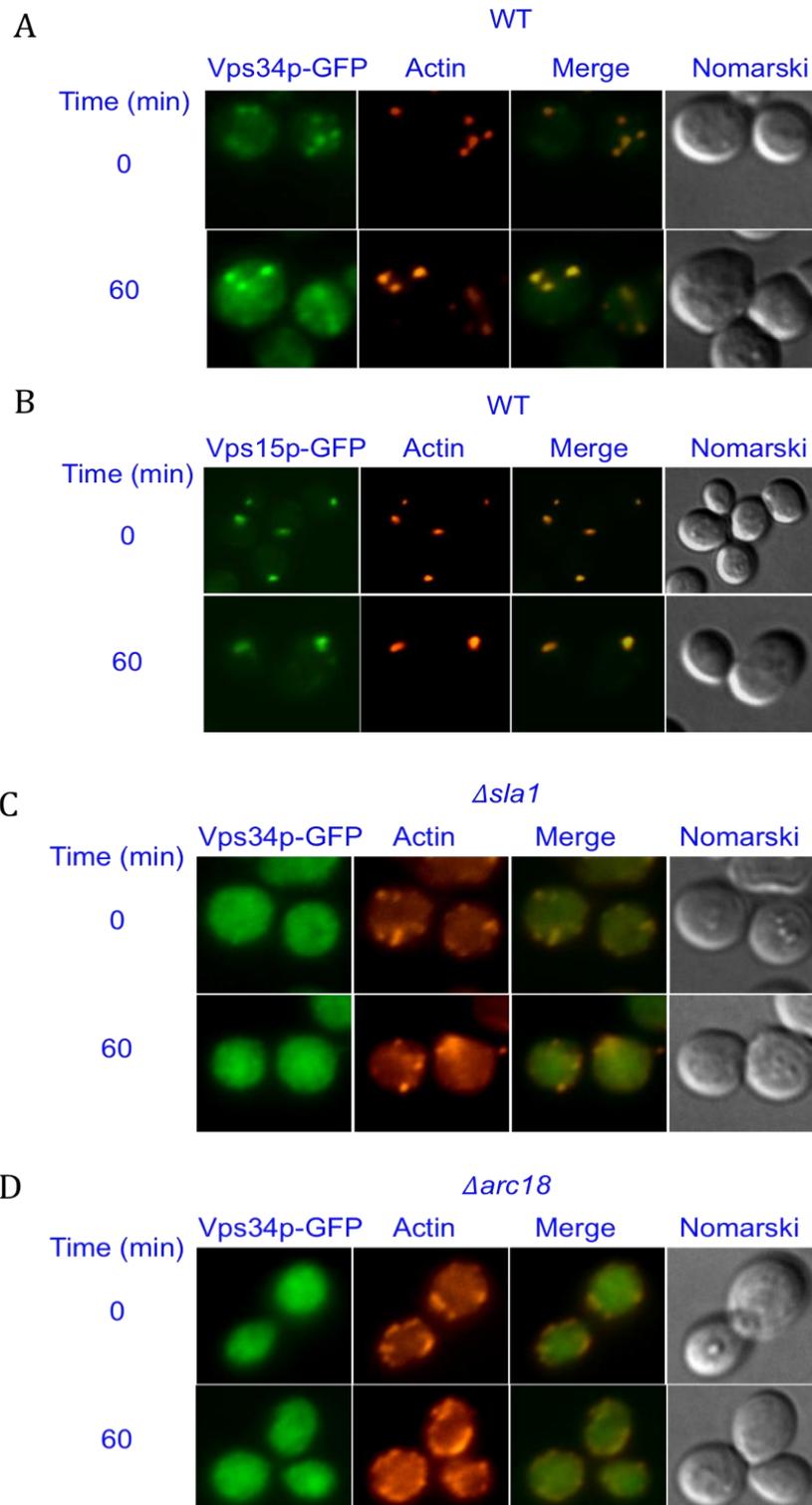
**Figure 2.** FBPase inactivation in wild type and in cells lacking *VPS34* and *VPS15*. Wild type, the  $\Delta vps34$  and  $\Delta vps15$  strains were glucose starved for 3d and re-fed with fresh glucose for 0 and 60 min. FBPase activity was measured. FBPase activity is expressed as the production of fructose-6-phosphate ( $\mu$ moles) per min per mg protein.



**Figure 3.** FBPase distribution to Vid vesicles is affected in cells lacking *VPS34* or *VPS15*. Wild type, cells lacking *VPS15* or *VPS34* genes were glucose starved for 3d and shifted to glucose for 20 min. Cell lysates were subjected to differential centrifugation to separate Vid vesicle enriched (P200) fraction and the cytosol (S200) enriched fraction. FBPase levels in the P200 and S200 fractions were determined.



**Figure 4.** Vps34p is enriched in the P100 fraction in wild type cells. Wild type cells expressing Vps34p-V5-His6 and wild type cells expressing Abp1p-cherry were glucose starved for 3d. Cell lysates were subjected to differential centrifugation. Levels of Vps34p-V5-His6, Abp1p-cherry and actin in P1, P13, P100, P200 and S200 were determined.



**Figure 5.** Vps34p distribution to actin patches is dependent on *SLA1* and *ARC18* genes. Wild type cells expressing Vps34p-GFP (A), wild type cells expressing Vps15p-GFP (B),  $\Delta sla1$  cells expressing Vps34p-GFP (C) and  $\Delta arc18$  cells expressing Vps34p-GFP (D) were glucose starved for 3d and shifted to glucose for 0 and 60 min. The distribution of GFP and actin was visualized by fluorescence microscopy.

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

*Innate Immune Responses to the Malaria Parasite* - Malaria is a major public health crisis around the world, affecting ~40% of the population and killing 2–3 million people annually. Currently, malaria is spreading rapidly due to drug resistance. A significant number of people from non-malaria regions (military and business personnel, diplomats and visitors) are also at increased risk and are particularly vulnerable to severe or even fatal forms of the disease because of their non-immune status. Therefore, novel drugs/therapeutics and/or vaccines are needed urgently. We propose to study cell signaling mechanisms in the innate immune responses produced by the host in response to malaria parasite infection and the regulation of innate immune responses. The knowledge gained by the proposed studies should prove to be valuable in developing novel immunotherapeutics and/or vaccines for combating malaria.

#### **Duration of Project**

1/1/2007 - 6/30/2010

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

*Chromatin Structure in Silent and Active URA3 Gene In Vivo* - We have developed a novel technique: EM-assisted nucleosome interaction capture (EMANIC) for studying nucleosome interactions in condensed chromatin in vitro. The purpose of this work is to extend this technique to study chromatin organization of a single gene (URA3) *in vivo*. URA3 is one of the most widely used genetic models. This gene has been placed in a yeast *S.cerevisiae* plasmid under control of silencing (HML) and activation (STAR) genetic regulators and functionally tested.

#### **Duration of Project**

4/1/2007 -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 8: Project Title and Purpose**

*The Effects of Sleep Deprivation on Addiction and Relapse* - The proposed studies will evaluate whether chronic sleep deprivation (SD), akin to levels commonly experienced by humans,

facilitates addiction and relapse to cocaine in rats, and the underlying neural mechanisms by which those effects are mediated.

### **Duration of Project**

4/1/2007 – 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 Infrastructure Project 9: Project Title and Purpose**

*Research Infrastructure: Renovations to Chandlee and Wartik Laboratories* - The demand for campus-wide core facilities has increased significantly in the last five years, particularly the Nucleic Acid Sequencing facility, the DNA Microarray facility, and the Biotechnology teaching labs. These facilities are currently housed in the 2<sup>nd</sup> floor of the Wartik laboratory and will be moved to the 4<sup>th</sup> floor of Chandlee. The renovation of Chandlee will allow researchers easy access to the new facilities. The 2<sup>nd</sup> floor of Wartik will then be renovated and become a state-of-the-art facility to house new faculty in Genomics and Proteomics, and will cater to the structural biology research needs of more than fifty investigators from five different colleges.

### **Duration of Project**

7/1/2007 - 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 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 10: Project Title and Purpose**

*Analyzing Hepatitis B Virus Pathogenesis* - The goal of this project is to investigate and characterize the effect of a novel anti-Hepatitis B virus (HBV) agent (shRNA-458) on HBV replication, specifically focusing on its effect on nuclear HBV covalently closed circular DNA (cccDNA) pools, which are responsible for HBV transcription and persistent HBV infections and the recycling of HBV nucleocapsids back into the nucleus. The efficacy of shRNA-458 will be compared to those of nucleoside and nucleotide analogues currently used to treat chronic HBV infections and other shRNA sequences targeting a different region of the HBV genome (HBV 2/20) other than that targeted by shRNA-458. The efficacy of shRNA-458, when administered at multiple doses and in conjunction with nucleoside and nucleotide analogues or HBV 2/20, will also be investigated.

## **Duration of Project**

1/1/2007 – 6/30/2010

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

*Dopamine Mediated Calcium Dynamics in Schizophrenia* - Schizophrenia is a devastating neuropsychiatric disorder, the etiology of which is unknown. Dysfunction in dopamine neurotransmitter signaling has been implicated as an important component of the disease. However, no defects in the cellular receptors, which transduce the dopamine signal, have been found. This project will identify components of the dopamine signaling system that may be altered in schizophrenia. We have recently identified a novel dopamine receptor interacting protein called TRPC (transient receptor potential channel) that connects dopamine and calcium signaling pathways in cells. This channel has been shown to be required for nicotine dependent behaviors in *C. elegans*, a model genetic system. In this project, we seek to validate the dopamine receptor-TRPC interaction and to understand the significance of this interaction for cellular function.

## **Duration of Project**

4/1/2007 - 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>.

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

*Research Infrastructure: Center for the Treatment, Prevention, and Cure of Cancer* - To design and construct a new building for the Penn State Cancer Institute that will bring together patient care, and basic and translational research under one roof to enhance our ability to provide comprehensive, coordinated cancer care. The new facility will allow top researchers to work together to create new therapies and to bring them quickly into state-of-the-art clinical care for the patients of central Pennsylvania. The funds requested are in addition to tobacco settlement Year 2005 funds allocated, since the entire project cost is estimated to be ~\$100 million.

## **Duration of Project**

7/1/2007 – 6/30/2010

## **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 13: Project Title and Purpose**

*Role of Leucine Metabolism in Leucine Signaling* - Recent evidence has suggested that increasing protein in the diet or some high protein diets promote weight loss through unexplained mechanisms. A component of protein called Leucine appears to be responsible. To examine the effect of elevating leucine, we knocked out an enzyme (BCATm) that begins leucine metabolism. The resulting mice had high plasma leucine concentrations in most tissues except their brain. More importantly, they were lean (about 60% less adipose tissue) and robustly resistant to obesity with improved glucose tolerance and insulin sensitivity. They actually eat more food. Our research is now focused on the molecular mechanisms explaining how knockout of this gene and increasing leucine brings about this phenotype. It should be possible to make blockers for this enzyme to bring about the same effects in humans.

## **Duration of Project**

4/1/2007 - 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 14: Project Title and Purpose**

*Mechanisms of TGF-beta Production in Human Cancer Cells* - Tumor cells that are resistant to the growth inhibitory effects of TGF $\beta$  can still secrete TGF $\beta$ , which enhances tumorigenesis. It is advantageous to block this secreted TGF $\beta$  in late-stage tumors. These studies will investigate the role of a novel component (km23) in controlling TGF $\beta$ 1 production using a novel, multidimensional approach. The results of the studies should reveal significant differences between human colon carcinoma cells (HCCCs) and untransformed epithelial cells (UECs) in the altered compartmentalization of signaling complexes, as well as in the differential utilization of signaling components. The goal of this project is to reduce production of a growth factor that enhances the spread of colon cancer. The results should lead to the development of novel approaches to treat colon cancer. The tobacco funds for this project will be used as bridge

funding to enable a Senior Research Associate and a Postdoctoral scholar gather additional data in response to reviewer's comments for the renewal of our NIH grant # NIH 2 RO1 CA 090765.

### **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 15: Project Title and Purpose**

*Mechanisms of Cross Priming In Vivo* - CD8+ T cells can protect against many pathologies, including viral, bacterial and fungal infections, as well as against many different kinds of tumors. These cells are only triggered after recognition of pathogen- or tumor-derived peptides in complex with MHC Class I molecules. The processes by which these peptide-MHC complexes are generated have been investigated in vitro, but the contribution of different pathways has not been investigated in vivo. Information about the mechanisms involved in peptide-MHC complex generation is essential for inclusion of the most efficient and effective formulation of antigen in vaccine preparations. In this project, those critical components of one antigen processing pathway, the cross priming pathway, will be identified.

### **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 16: Project Title and Purpose**

*Proteomics of Substance Abuse* - Ethanol abuse and alcoholism remain very serious societal problems producing a tremendous toll in terms of loss-of-life, adverse health effects, and lost productivity. A significant problem is the lack of a sensitive and specific clinical test to diagnose alcohol abuse either in the general population or within selected groups of individuals such as recovering/relapsing alcoholics. The goal of this project is to screen for signature biomarkers in a well-controlled and extensively documented non-human primate model of self-administration in order to develop diagnostic markers of excessive alcohol consumption.

## **Duration of Project**

4/1/2007 - 6/30/2007

## **Summary of Research Completed**

This project was dropped prior to the expenditure of any grant funds.

## **Research Project 17: Project Title and Purpose**

*Positive Selection-dependent Skin-homing of Fetal Thymic  $\gamma\delta$  T Cells* - To fight efficiently against cancers, infection and other diseases, immune cells reside in specific tissues where they function uniquely to protect the local tissues. Disruptive localization of the immune cells in proper tissues renders a body susceptible to diseases. Therefore, understanding molecular mechanisms of development and localization of these immune cells in specific tissues is important for our effort to improve our health. The purpose of this project is to determine molecular and cellular events regulating the developmental process of skin-specific  $\gamma\delta$  T cells, an important class of immune cells.

## **Duration of Project**

1/1/2007 – 12/31/2010

## **Project Overview**

$\gamma\delta$  T cells are a unique class of immune cells that plays important roles in tumor surveillance, microbial immunity and others. Many  $\gamma\delta$  T cells preferentially reside in epithelial layers of various tissues underlying external and internal surfaces of the body, such as skin, lung and intestines, where they function as the first line of defense. The murine skin-specific intraepithelial  $\gamma\delta$  T cells (sIELs) are some of the most representative tissue-specific  $\gamma\delta$  T cells, which play important roles in the prevention of cutaneous tumors, regulation of skin-allergic responses and others. Nearly all sIELs express identical V $\gamma$ 3/V $\delta$ 1 g $\delta$ T cell receptors (TCR) and originate from the fetal thymus. Recently, it was discovered that the skin-specific  $\gamma\delta$  T cell precursors generated in the fetal thymus underwent a unique selection process that renders them unique homing properties, which may determine their specific migration and localization into the skin. These findings suggest that central selection in the thymus determines their peripheral tissue distribution, a novel conceptual advance in understanding the development of the tissue-specific lymphocytes. To test and define this hypothesis, the project proposed here will include two specific aims to study molecular and cellular events that link fetal thymic selection of  $\gamma\delta$  T cells with their skin-specific tissue distribution.

**Specific Aim 1: To dissect TCR/ligand interaction-mediated fetal thymic  $\gamma\delta$  T cell selection, in relationship with its unique homing properties and skin-specific tissue distribution.**  $\gamma\delta$  TCR transgenic mice with known ligands will be employed to investigate how TCR/ligand engagement mediates selection of fetal thymic  $\gamma\delta$  T cells and determines their homing properties and unique peripheral tissue distribution in the skin.

Specific Aim 2: To determine the roles of CCR10 in sIEL development using CCR10-knockout/EGFP-knockin mice. Chemokine receptor CCR10 is upregulated in the positively selected fetal thymic sIEL precursors. Constitutive expression of its ligand CCL27 in the skin suggests a potential role of CCR10 as a skin-homing receptor for the sIEL precursors. CCR10-knockout/EGFP-knockin mice will be generated and used to determine the role of CCR10 in sIEL development.

### **Principal Investigators**

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### **Other Participating Researchers**

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

This project will improve the fundamental understanding of genesis of the skin-specific  $\gamma\delta$  T cells. Furthermore, as the first approach to investigate differential homing processes of various immune cell populations, this project will serve as a paradigm for understanding specific migration and localization of other tissue-specific immune cells. By identifying molecules that regulate migration and localization of  $\gamma\delta$  T cells in the skin, we might be able to design a better strategy to target these important immune cells to specific tissues to fight against diseases.

### **Summary of Research Completed**

We have submitted all our major findings for publication in this period. Therefore, our main work in this period has been to finish the experiments addressing reviewers' specific questions regarding our papers. By the end of the report period, four papers have been published.

### **Research Project 18: Project Title and Purpose**

*In Vivo Brain Tissue Iron Measurement with R2\* Mapping in Alzheimer's Disease* - To develop quantitative *in vivo* MRI methods to evaluate brain iron associated with early Alzheimer's disease (AD) pathology.

### **Duration of Project**

4/1/2007 - 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 19: Project Title and Purpose**

*Combined Effects of a Hypoxia Selective Prodrug and Herceptin to Breast Cancer* - Clinical studies suggest that the addition of Herceptin to anthracycline-based chemotherapy, such as doxorubicin, increases its clinical benefit and reduces the recurrence rate by approximately 50%. Although doxorubicin is very effective against breast cancer, the development of heart toxicity and drug resistance by doxorubicin leads to a need in finding safe alternatives. A recently developed novel anticancer agent, acridiol-sulfoxide, may selectively kill solid tumor cells by being converted into its active form in the oxygen deficient environment of a tumor. Therefore, the goal of current study is to establish the use of acridiol-sulfoxide as an alternative to doxorubicin, in combination with Herceptin to treat breast cancer in *in vitro* cell culture models.

### **Duration of Project**

8/9/2007 – 12/31/2009

## **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement 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 20: Project Title and Purpose**

*Nitroaromatics and Breast Cancer* - The cause of most breast cancers remains unknown. In addition to genetic predisposition, exposure to chemical carcinogens in the diet, tobacco, and polluted air has been implicated as a factor in the development of breast cancer. A representative example is the environmental pollutant 6-nitrochrysene (6-NC) which is normally produced from incomplete combustion of organic compounds such as diesel, gas, kerosene and liquid petroleum; 6-NC is a powerful carcinogen in breast tissues of rodents. Studies have shown that 6-NC can be detected in human blood and can damage DNA in breast tissue of animals. The goal of this study is to use state of the art technology, liquid chromatography-tandem mass spectrometry (LC-MS-MS), to identify and quantify the levels of DNA damage induced by this environmental pollutant initially in animals, and eventually in humans.

### **Duration of Project**

8/9/2007 – 6/30/2010

## **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 21: Project Title and Purpose**

*Modeling Rapid Osteoblastic Bone Metastases* - The effects of intermittent parathyroid hormone (PTH) on the establishment of *osteoblastic* bone metastases are unknown. The work to be completed with the requested funds is to establish that increased bone formation as a consequence of intermittent PTH treatment, will facilitate colonization of human prostate cancer (PCa) cells in bone. Specifically, we plan to test two hypotheses: 1) that intermittent PTH treatment will facilitate the establishment and colonization of human osteoblastic PCa cells in bones of mice; and 2) that human osteoblastic PCa colonization of murine bone requires a bone environment that favors bone-formation.

### **Duration of Project**

8/9/2007 – 12/31/2009

## **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement 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 22: Project Title and Purpose**

*Molecular Functional Studies of Neurog3 Gene Variants* - The goals of the present study are 1) to establish in vitro the impact of NEUROG3 rare and common variants on NEUROG3 transcription regulation and downstream target gene modulation; and 2) to establish the potential role in the pathogenesis of T2D of NEUROG3 promoter and regulatory variants, NEUROG3 haplotypes and diplotypes.

### **Duration of Project**

8/9/2007 – 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>.

### **Research Project 23: Project Title and Purpose**

*Role of Excess Nutrients in Diabetic Retinopathy* - Interactions between dysregulated glucose and amino acid metabolism caused by Type 1 diabetes lead to inflammation followed by retinal cell death. Our goal is to evaluate these toxic processes and find out why they occur. Therefore, the study will facilitate development of drugs needed for reversal of toxicity. For example, interfering with the inflammatory response using the anti-inflammatory agent, minocycline, lowers neuronal cell death in diabetic rat retinas. Lowering formation of reactive oxygen species may also be therapeutic. These interventions will be studied for their potential in treating patients.

#### **Duration of Project**

8/9/2007 – 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 24: Project Title and Purpose**

*HFE Polymorphisms on the Chemotherapy and Patient's Outcome of the Brain Tumors* - This project will demonstrate that individuals with brain tumors that carry an HFE polymorphism have a worse outcome than those with wild type HFE, and provide a cell culture model in which to identify the mechanisms by which HFE polymorphisms affect cell behavior and provide a first line in which to test intervention strategies.

#### **Duration of Project**

8/9/2007 – 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 25: Project Title and Purpose**

*Mu Opioid Receptor in Addiction* - The mu-opioid receptor (MOR) mediates most of the actions of morphine and other clinically relevant analgesics as well as drugs of abuse such as heroin. Recent studies have identified a number of MOR interacting proteins (MORIPs) including filamin A, protein kinase C interacting protein, and periplakin, suggesting that the MOR is organized within a supramolecular signaling complex. Through the combined proteomic and

functional studies outlined in this project, we will identify and characterize a set of novel MORIPs. Identification of these MORIPs will allow us to test our hypothesis that MORIPs play important roles in regulating MOR biogenesis and MOR-mediated signal transduction, as well as providing new insight into the etiology of opioid dependence (OD).

### **Duration of Project**

8/9/2007 – 12/31/2010

### **Project Overview**

The mu-opioid receptor (MOR) belongs to the superfamily of 7 TM G-protein coupled receptors (GPCRs) and mediates most of the actions of clinically relevant analgesics as well as drugs of abuse such as heroin. Recent studies have identified a number of MOR interacting proteins (MORIPs). These MORIPs have been shown to play a role in regulating MOR trafficking and signal transduction. In this project, we will identify and characterize a set of novel MORIPs. Identification of these MORIPs will allow us to test our hypothesis that MORIPs play important roles in mediating opioid dependence (OD). MORIPs also represent novel targets for the development of drugs designed to treat addictive behaviors.

**Aim 1. Identification and Characterization of Mu-Opioid Receptor Interacting Proteins.** Our primary goal is to identify novel MORIPs and elucidate the role these proteins play in regulating MOR-mediated signaling. We will carry out a split-ubiquitin yeast two-hybrid (Y2H) screen to identify novel MORIPs. This method is permissive for detection of interactions involving integral membrane proteins. In a complementary approach, we will perform a conventional Y2H screen to identify additional candidate MORIPs. Together, these approaches should allow us to discover many of yet unidentified components of the MOR signaling complex.

**Aim 2. Validation of MOR Interacting Proteins.** The major goal in Aim 2 is to validate the candidate MORIPs discovered in the Y2H screens. Several independent criteria will be used to validate protein-protein interactions including cellular colocalization, pull-down and coimmunoprecipitation. Deletion mapping will permit identification of sites within the proteins that are necessary for the interactions to occur. Validation of novel MORIPs will serve to inform the functional studies outlined in Aim 3.

**Aim 3. Functional Analysis of MOR/MORIP Interactions.** This aim is focused on elucidating the role that MORIPs play in regulating MOR-mediated signaling. We will examine the requirement for the MOR/MORIP interaction in regulation of MOR function including receptor trafficking, endocytosis, internalization and desensitization. To do this, we will determine whether disruption of the MOR/MORIP interaction (either by expression of a dominant negative form of the interactor, or a competing peptide against one of the protein binding domains) affects trafficking or other functional properties of the MOR. These results should provide new insight into the regulation of MOR-mediated signaling and drug dependence.

## **Principal Investigator**

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## **Other Participating Researchers**

Jay Jin – employed by the Penn State College of Medicine

## **Expected Research Outcomes and Benefits**

The major goal of this project is to identify a novel cohort of mu-opioid receptor (MOR) interacting proteins (MORIPs) and elucidate the functional role of MOR/MORIP interactions in regulating the multiple processes involved in MOR biogenesis and signaling. Through the identification and characterization of novel MORIPs, this project will help establish a new platform for defining cellular mechanisms of MOR signaling in the brain; as well contribute to our understanding of the underlying basis of opioid tolerance and opioid dependence (OD).

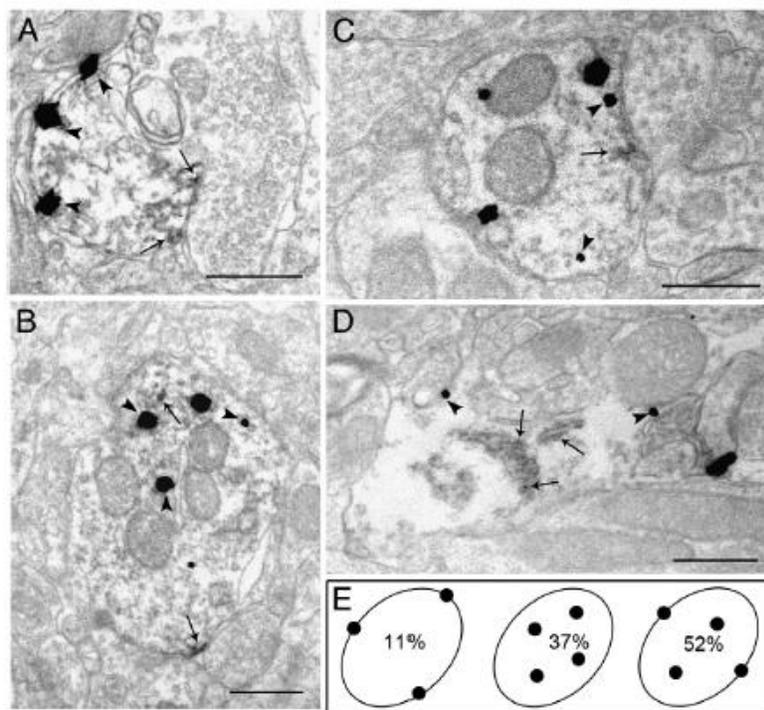
In the long term, the line of research described in this project may not simply be an approach for learning more about MOR biogenesis and signaling, but a fruitful strategy for identifying candidate risk genes for OD as well. For example, each MORIP we identify and validate will represent a candidate gene to be studied in paradigms of OD and withdrawal through OD genetic association studies that we will carry out in collaboration with Penn. MORIPs that satisfy all validation inclusion criteria may be further characterized by generating murine null mutants as a means to understand the role of novel MORIPs in opioid-related murine phenotypes.

The identification of MORIPs as modulators of MOR-mediated signaling holds the promise of opening exciting new areas relating the function of MORIPs with behavior and drug dependence. In particular, because of the well-established central role of the MOR in the rewarding actions of opioids, the identification of novel MORIPs may provide conceptual breakthroughs in our understanding of opioid tolerance and OD, as well as the development of novel therapeutic interventions. Insight into the functions of known as well as novel MORIPs should also enhance our ability to improve the efficacy of therapeutics designed for the treatment of OD.

## **Summary of Research Completed**

We have previously identified GPR177 (now officially known as Wntless or WLS), the mammalian ortholog of *Drosophila* Wntless/Evi/Sprinter, as a novel mu-opioid receptor (MOR) interacting protein. WLS is a transmembrane protein pivotal to mediating the secretion of Wnt signaling proteins. Wnt proteins, in turn, are essential in regulating neuronal development, a phenomenon inhibited upon chronic exposure to MOR agonists such as morphine and heroin. Our previous studies provided evidence that WLS and MOR are co-localized in the mouse dorsolateral striatum; however, the nature of this interaction was not fully elucidated.

During this grant period, we examined cellular substrates for interactions between WLS and MOR using a combined immunogold-silver and peroxidase detection approach in coronal sections in the dorsolateral segment of the striatum. As shown in Fig. 1, semi-quantitative analysis of the ultrastructural distribution of WLS and MOR in striatal somata and in dendritic processes showed that, of the somata and dendritic processes exhibiting WLS, 32% contained MOR immunolabeling while for profiles exhibiting MOR, 37% also contained WLS immunoreactivity. WLS-labeled particles were localized predominantly along both the plasma membrane and within the cytoplasm of MOR labeled dendrites. Somata and dendritic processes that contained both WLS and MOR more often received symmetric (inhibitory-type) synapses from unlabeled axon terminals. To further define the phenotype of GPR177 and MOR-containing cellular profiles, triple immunofluorescence detection showed that GPR177 and MOR are localized in neurons containing the opioid peptide, enkephalin, within the dorsolateral striatum. The results provide an anatomical substrate for interactions between MOR and its interacting protein, GPR177, in striatal opioid-containing neurons that may underlie the morphological alterations produced in neurons by chronic opiate use.



**Fig. 1** – Electron photomicrographs showing immunogold-silver labeling for WLS and immunoperoxidase labeling for MOR in the striatum as well as an example where markers are reversed. (A–C) Dually labeled GPR177 and MOR-dendrites containing WLS (arrowheads) along the plasma membrane only (A), within the cytoplasm only (B) and both within the cytoplasm as well as the plasma membrane (C). Arrowheads point to WLS while arrows point to MOR. (D) Example of tissue sections processed where the detection methods were reversed. In this electron photomicrograph, WLS is labeled using immunoperoxidase detection (arrows) while MOR is indicated by immunogold-silver labeling (arrowheads). (E) Schematic diagrams illustrate the distribution of WLS in MOR-labeled dendrites when WLS is localized to the plasma membrane only, within the cytoplasm only, or both within the cytoplasm and the plasma membrane. A higher percentage of MOR-labeled dendrites exhibited WLS labeling when WLS

was present both on the plasma membrane as well as within the cytoplasm. m, mitochondria; ut, unlabeled terminal. Scale bars, 0.5  $\mu$ m.

### **Research Project 26: Project Title and Purpose**

*Role of TGF $\beta$  in Microtubule Dynamics* - A major significance of the project relates to our finding that 42% of ovarian cancer patients have alterations in the motor protein subunit we discovered, termed km23, with no such alterations in normal tissues. Such a high alteration rate in ovarian cancer suggests that km23 plays an important role in this disease. The novelty of the proposed studies relates to the plan to investigate trafficking of specific TGF $\beta$  signaling components in relation to the km23 motor subunits, and to determine how these events are altered by ovarian cancer mutations. The overall goal of this project is to better understand how a critical growth regulator sends its signals to stop growth, and how these events are altered in human cancer. The results will assist with the development of novel anti-cancer agents to treat ovarian and other cancer types.

### **Duration of Project**

8/9/2007 – 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 27: Project Title and Purpose**

*Novel Therapeutic Strategies to Treat Malaria* - Malaria causes 300-500 million new infections, 1-3 million deaths, and 1-4% loss of gross domestic product annually in Africa. The majority of its victims are young children and pregnant women. One child under five dies every 20 seconds of malaria and survivors frequently suffer brain damage and learning disabilities. There are few available anti-malarial drugs, no vaccines and embarrassingly few drugs in the anti-malarial drug pipeline. Thus, there is an unprecedented need to develop new drugs to treat malaria. Malaria parasites express two proteins, called 'ion channels', which are members of a large family of proteins that are essential for the survival of all living organisms. Preliminary findings indicate that targeting the malarial ion channels with drugs kills parasites *in vitro*. The purpose of this research is to develop a deeper understanding of the role ion channels play in the normal physiology of the malaria parasite and their potential as anti-malarial drug targets.

### **Duration of Project**

8/9/2007 – 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 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 28: Project Title and Purpose**

*Center for Functional Genomics* - Our understanding of biological systems is becoming increasingly sophisticated, to the point where additional learning is being hampered by our inability to grasp the vast warehouse of knowledge. This bottleneck is being magnified exponentially as we develop increasingly more powerful means of high-throughput data collection. Moreover, teaching this complexity to students at all levels (K-12, college, and beyond) becomes increasingly more of a challenge as tech-savvy students expect more “user-friendly” learning environments. The purpose of this project is to develop a bioinformatic browser that helps students, scientists, and lay people understand the complexity of the cellular nucleus. This project will also contribute to and draw from knowledge retained in the browser.

### **Duration of Project**

8/9/2007 – 12/31/2010

### **Project Overview**

The Center for Functional Genomics has three broad objectives. The first objective is to construct an internet browser that assimilates a broad range of genomic data so that relationships can be readily visualized. The browser is expected to have varying levels of sophistication such that the information would be of interest to students at all levels. Students in the primary grades would primarily use it as a “fun” way to explore. Students and laypersons at upper levels would use it to understand concepts about how cells are regulated at the molecular level. Scientists would use it to conceptualize complex relationships among gene regulatory molecules for purposes of discovery and modeling. Specific aims in this first objective include the production of a graphical user interface, and tools to retrieve, deposit, and model a variety of genomic information.

The second objective is to provide raw experimental data to upload into the browser that the information can be displayed either quantitatively or graphically through icons. Personnel associated with the project will conduct genomic experiments in a number of collaborative laboratories and upload the data sets into the browser.

The third objective is to use the browser as a discovery platform. Personnel associated with the project will use the browser to discover novel relationships among hordes of genomic information. These relationships will be formulated into a testable hypothesis that will be directly investigated in a collaborative laboratory. Funds associated with the project will be used to seed these goals, with the intent that federal support will ultimately carry the bulk of the financial needs of the project.

### Specific Aims:

- 1) Develop a web-based browser to visualize genomic data.
- 2) Experimentally test the prediction that gene regulatory proteins bind to fixed locations at the beginning of genes throughout the genome of model organisms (yeast initially, then *Drosophila* and human).
- 3) Experimentally test whether regulatory proteins that co-localize to gene promoters are functionally dependent upon each other.
- 4) Use the data generated in aims 2 and 3 that has been uploaded into the browser developed in aim 1 to experimentally validate on individual genes the observed relationships.

### Principal Investigator

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### Other Participating Researchers

Istvan Albert, PhD, Anton Nekrutenko, PhD, Stephan C. Schuster, PhD, Ross C. Hardison, PhD, David S. Gilmour, PhD, Song Tan, PhD, Joseph C. Reese, PhD and Yanming Wang, PhD – employed by Penn State University

### Expected Research Outcomes and Benefits

Specific outcomes of this project include a genome browser decorated with icons that are representative of genomic information. Such genomes include human and those of model organisms. Tracks of information include the location of nucleosomes (building blocks of chromosomes), locations of genes, locations of gene regulatory proteins and DNA regulatory sites. The browser will be navigable via one-dimensional scrolling to move along the one-dimensional string of DNA. Later versions of the browser are expected to include plug-ins that allows the genomic information to be represented as actual three-dimensional structures rather than the use of schematic icons as proxies. The plug-in will allow navigators to move around the structures in a virtual 3-D environment. A substantial portion of the data represented in the browser is expected to be generated through the project. Specific testing of hypotheses arising from the modeling will allow the hypothesis to be validated or discarded.

The benefit of this work to society is that it provides an educational tool for all people to understand the inner workings of a cell, which is critical for making informed decisions in society. Second, it provides scientists with a means of distilling a tsunami of data into simple concepts that the human brain is more adept at understanding. This will facilitate further discovery in biological systems, in particular gene regulatory systems. Understanding how genes are regulated is critical towards creating strategies to repair damaged genes or mis-regulated genes.

## Summary of Research Completed

During this reporting period the work described previously has been accepted in the journal *Molecular Cell*, which is considered to be one of the most pre-eminent journals in this field. Another aspect of this work will be published in the journal *Molecular and Cellular Biology* in January 2011. In addition, we have prepared a manuscript describing Bioscape that we expect to submit for publication soon. A manuscript is in preparation pertaining to collaborative studies between Pugh and Gilmour in *Drosophila*, and between Pugh and Wang in mammalian systems. Much of the past 6 months have been devoted to further development of the high resolution genome-wide mapping.

Previously, we created and verified the genotype of 88 mutant strains that represent 4 different deletions in 22 different strains containing TAP-tagged factors. Subsequently, we have performed over 80 ChIP-seq experiments from the *gcn5D*/TAP-tag subset of strains. In order to minimize cost and maximize throughput, this ChIP enriched material was multiplexed during library preparation for the ABI SOLiD sequencer. The ability to conduct this number of ChIP-seq experiments was made possible through the availability of ARRA funds. Otherwise we suspect that only 10% of this number would have been done. ARRA allowed us to hire two additional technicians, additional bioinformatic support, and additional equipment to move the project along with increased tempo. Data has now been collected for two biological replicates and under normal vs. heat shock conditions, totaling 80 ChIP-seq data sets. The data is currently being analyzed using an established bioinformatics pipeline in our lab. Table 1 below summarizes our progress in this area.

We developed a novel assay to examine whether a factor binds to nucleosome in vivo. This study demonstrated an interaction of Bdf1 with nucleosomes in vivo. This interaction has been shown to be dependent on NuA4-directed histone acetylation but independent of TAF1. In an effort to mechanistically define Bdf1-nucleosome interactions, we have now tested the contributions of H3 and H4 histone tails, histone acetyltransferases (*Gcn5*, *Hat1*, and *Sas2*), H2A.Z (*Htz1*), and the H2A.Z remodeler (*Swr1*) (Fig. 1). In addition to *Esa1* (NuA4), we found Bdf1-nucleosome binding to be dependent on *Gcn5* (SAGA, Fig. 1A) and the H4 tail (Fig. 1B). Since the Bdf1-nucleosome interaction is dependent on the H4 tail but not the H3 tail, we hypothesize that *Gcn5* (SAGA) regulates Bdf1-nucleosomal binding through acetylating Bdf1 rather than the H3 lysines and that H4 acetylation by *Esa1* (NuA4) then helps recruit Bdf1.

Previously, we examined how nucleosome positions are affected genome-wide in several SAGA deletion mutants. To determine which SAGA subunits interact with nucleosomes, we have now tested 7 different SAGA subunits (*Ada2*, *Chd1*, *Gcn5*, *Spt3*, *Spt7*, *Taf6* and *Ubp8*) using our nucleosome interaction assay (Koerber et al., 2009). Of those tested, only *Gcn5* showed an interaction with nucleosomes.

This work is important because it is helping to elucidate how assembly of the transcription machinery depends upon other factors on a genomic scale. Knowledge of global mechanisms by which this assembly occurs is key to understanding how diseases arise through gene mis-expression.

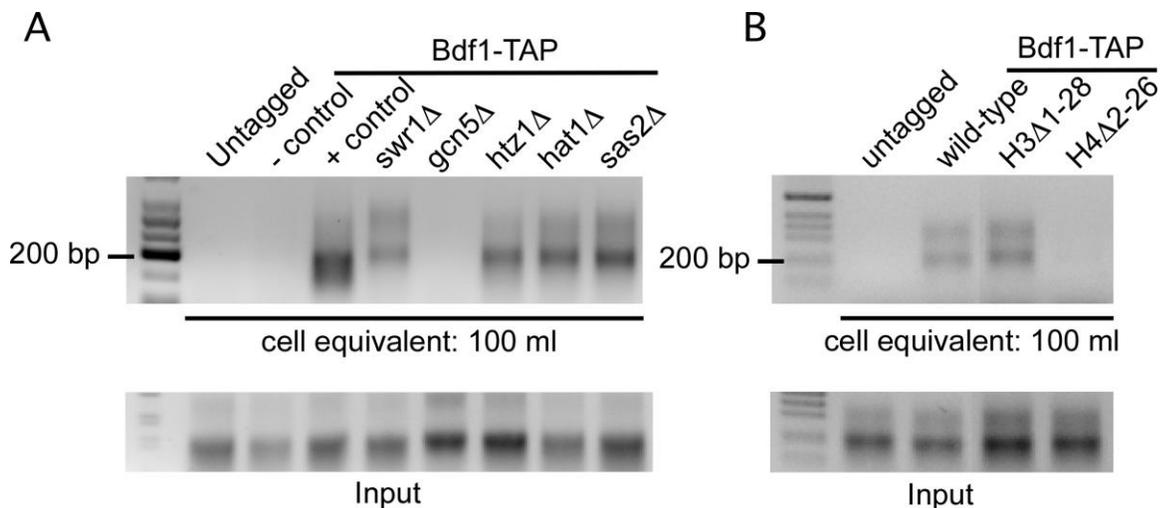
Table 1. Factor mapping experiments (sample set of the total strains created)

Condition	IP	Why? <sup>a</sup>	Data collection status	Samples <sup>b</sup>
Wild type	TAP strains	Reference state	Complete	16
<i>gcn5Δ</i>	Esa1-TAP	H3 histone acetylase & H4 histone acetylase	Complete	4
<i>gcn5Δ</i>	Srb5-TAP	Histone acetylase & Mediator	Complete	4
<i>gcn5Δ</i>	Rgr1-TAP	Histone acetylase & Mediator	Complete	4
<i>gcn5Δ</i>	Swr1-TAP	Histone acetylase & H2A.Z remodeler	Complete	4
<i>gcn5Δ</i>	Vps72-TAP	Histone acetylase & H2A.Z remodeler	Complete	4
<i>gcn5Δ</i>	Rsc9-TAP	Histone acetylase & positive remodeler	Complete	4
<i>gcn5Δ</i>	Swi3-TAP	Histone acetylase & positive remodeler	Complete	4
<i>gcn5Δ</i>	Snf2-TAP	Histone acetylase & positive remodeler	Complete	4
<i>gcn5Δ</i>	Isw2-TAP	Histone acetylase & negative remodeler	Complete	4
<i>gcn5Δ</i>	Itc1-TAP	Histone acetylase & negative remodeler	Complete	4
<i>gcn5Δ</i>	loc3-TAP	Histone acetylase & negative remodeler	Complete	4
<i>gcn5Δ</i>	Tup1-TAP	Histone acetylase & co-repressor	Complete	4
<i>gcn5Δ</i>	Taf1-TAP	Histone acetylase & TFIID initiation	Complete	4
<i>gcn5Δ</i>	Spt3-TAP	Histone acetylase & SAGA initiation	Complete	4
<i>gcn5Δ</i>	Rpo21-TAP	Histone acetylase & RNA polymerase II	Complete	4
<i>gcn5Δ</i>	Rpb3-TAP	Histone acetylase & RNA polymerase II	Complete	4

<sup>a</sup>Addresses how the genomic distribution of the latter (IP'd factor) depends on the former (condition: deletion strain).

<sup>b</sup>Minimum of 2 biological replicates per parameter.

Figure 1. Bdf1-nucleosome binding is dependent on Gcn5 and the H4 tail.



## **Research Project 29: Project Title and Purpose**

*Regulation of Human Sebaceous Glands by 13-cis Retinoic Acid* - Severe acne can have profound psychological and physical morbidity for millions of affected patients. The overall goal of the research is to determine how isotretinoin, a potent drug for acne exerts its positive effects in the sebaceous gland by reducing sebum production and improving acne. Isotretinoin (13-*cis* retinoic acid) is the only drug effective against severe acne that targets each of the causative factors of this disease. 13-*cis* RA is also used in cancer chemotherapy. This drug however can cause birth defects. The specific goal of this project is to identify the active isomer or metabolite of 13-*cis* RA that induces apoptosis in human sebocytes. The experiments to be done with these tobacco settlement funds are critical to the overall success of a larger grant application to the NIH. This information can lead to the identification of potential therapeutic target sites that could be activated by drugs apart from 13-*cis* RA that would have an improved safety profile.

### **Duration of Project**

8/9/2007 – 12/31/2010

### **Project Overview**

Lack of understanding of the mechanism of action of 13-*cis* RA in the sebaceous gland has hampered the progress to find safer alternatives in the treatment of severe acne. Our preliminary data indicate that 13-*cis* RA induces apoptosis and cell cycle arrest in sebocytes. Gene array expression analysis in sebocytes and in human skin indicates that 13-*cis* RA induces genes involved in the innate immune response, including *lipocalin 2*, that encodes a multifunctional secretory protein called neutrophil gelatinase associated lipocalin (NGAL) that is involved in the formation of the kidney, the body's defense against bacterial pathogens and in cellular apoptosis. The major goal (specific aim) of the experiments done with tobacco settlement funds is to test the hypothesis that 13-*cis* RA acts by a metabolite or metabolites to induce apoptosis in human cells. Subaims are directed at determining how 13-*cis* RA differs from all-*trans* RA in mediating apoptosis. The findings of these studies not only have the potential to advance our understanding of retinoid action in acne, but can lead to advances in the understanding of these agents in cancer biology and in innate immunity as well.

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## Expected Research Outcomes and Benefits

The desired outcome for the overall research project is the discovery of the specific means by which 13-*cis* retinoic acid exerts its beneficial effects in suppressing function of the sebaceous gland and improving acne. It is unknown as to whether the drug itself or its metabolites mediate this suppression and whether these effects are mediated by retinoic acid receptors. *The studies done with tobacco settlement funds in this project should identify the isomer or metabolites of 13-cis RA that are responsible for inducing apoptosis in human sebocytes.*

In the context of the overall grant application to the NIH, if retinoic acid receptors are not involved, then it is possible that safer classes of drugs (those that do not cause birth defects) could be used to elicit the same beneficial effects as 13-*cis* retinoic acid. If the neutrophil gelatinase associated lipocalin protein (NGAL) mediates the effects of 13-*cis* retinoic acid on apoptosis or cell cycle arrest in sebaceous cells, then an alternative treatment for severe acne might involve therapies that are aimed at increasing the expression of the NGAL protein in the skin. The data generated in the overall project can lead to the identification of new therapeutic target sites in the treatment of severe acne. As such, this project has the potential to improve health status for hundreds of thousands of patients who are being treated for severe acne with 13-*cis* retinoic acid. Although this project studies the effects of 13-*cis* RA on cells from the sebaceous gland that relate to acne, the information gained from this study regarding the mechanism of action of 13-*cis* retinoic acid at a cellular level is also relevant to cancer chemotherapy since 13-*cis* retinoic acid is used to treat cancers such as neuroblastomas in children. This drug is also used in chemoprevention of cancers in patients with diseases or conditions characterized by frequent occurrence of skin cancers. As such, the data from this project including the segment funded by tobacco settlement funds has the potential to lead to advances in therapy of a common condition, acne, and serious conditions such as cancer.

## Summary of Research Completed

The IRB- approved recruitment of normal volunteers and patients scheduled to begin treatment with 13-*cis* retinoic acid (13cRA) has continued. The samples available for testing (Table 1) are not straight serum but rather a mix of 50% serum in RPMI medium due to required processing for other tests not covered under this grant. Testing was required to determine: (1) that the samples have detectable levels of retinoids, (2) the most appropriate commercially available internal standard (the desired pentadeuterated retinoic acid could not be produced by our core facility), (3) an appropriate matrix for the spiked standards of the standard curve, (4) an appropriate resuspension solution for the extracted samples, and (5) an extraction method for our serum:RPMI mix. A published extraction method (Kane, M, N. Chen, S. Sparks, and J. Napoli. 2005. Quantification of endogenous retinoic acid in limited biological samples by LC/MS/MS. *Biochem J.* 388: 363-369) was chosen as our starting point. The method involved the salting out of retinoic acid with 0.025M potassium hydroxide (KOH), hexane extraction to remove non-aqueous components of the mix, addition of 4M hydrochloric acid (HCl) to desalt the carboxylic acid and allow for a final extraction in hexane, which was dried down and the sample resuspended for processing.

1. Determination of Detectability of Retinoid Levels in Patient Serum: The first test run was performed using serum:RPMI mix from a patient 1 week post-treatment as we would hypothesize that those samples would have higher retinoid levels than pretreatment samples. All three retinoids [13cRA, 9-*cis* retinoic acid (9cRA), and all-*trans* retinoic acid (ATRA)] were detectable using our previously developed HPLC/MS/MS method. A test run was then performed using pretreatment patient serum and retinoid levels were barely detectable, which is desirable for use in the performance of a standard curve with spiked retinoids, for the determination of retinoid levels in patient samples. A bulk quantity of mixed serum:RPMI from a number of non-acne volunteers was pooled in order to produce a serum:RPMI supply of sufficient quantity to be used for all runs of patient samples.

2. Internal Standard (IS) Testing: Testing for an appropriate IS was initiated using retinol, one of several possible internal standards mentioned in the literature. Each test involved the extraction of a standard curve of pretreatment serum:RPMI spiked with 0, 0.3, 1, 2, 5, 10, 50, 100ng/mL of each of the three retinoids (13cRA, 9cRA, and ATRA). After testing several standard curves with increasing concentrations of retinol (10, 200, 500ng/mL), it was determined that retinol was not an appropriate choice for our samples and methods as it was barely detectable (Figure 1). Acitretin was then selected for testing and was determined to be suitable at 50ng/mL after first testing higher concentrations (Figure 2).

3. Determination of an Appropriate Matrix for the Standard Curve: Standard curves were run in pretreatment serum:RPMI mix, 4% bovine serum albumin (BSA) in phosphate buffered saline (PBS), or water. Use of a 4% BSA or water matrix would eliminate the need to perform back calculations to subtract low background levels of retinoids found in the matrix from standard curve data, a necessity if using a serum:RPMI matrix. Neither the 4% BSA nor the water was an appropriate matrix for our standard curve as the retinoids had a different profile in those matrices than in the serum:RPMI samples (data not shown). Pretreated serum:RPMI will be used and back calculations performed in accessing the standard curves.

4. Determination of an Appropriate Resuspension Solution for Extracted Samples: Final samples were resuspended in 100% acetonitrile (ACN), 70:30 ACN:water, 50:50 ACN:water, or 100% ACN followed by the addition of HPLC grade water for a final 50:50 mix. Initial resuspension in 100% ACN followed by the addition of water gave the sharpest peaks with good detection levels and was selected as our method of post extraction resuspension.

5. Determination of an Appropriate Extraction Method for Serum:RPMI Mix: After several test standard curves with the above mentioned extraction method, it was determined that a protein precipitation step should be included prior to extraction in order to clean-up the samples. ACN:methanol (MeOH) 50:50 was added to each sample in a ratio of 5 parts ACN:MeOH to 1 part straight serum, precipitate removed by centrifugation at 3,700 rpm for 15 minutes (min), supernatant was transferred to a new sample tube, dried down under nitrogen gas, resuspended in 200 $\mu$ L ACN, and 200 $\mu$ L water added, followed by the above mentioned extraction method. Final sample clarity was improved but still appeared to be slightly cloudy. After a literature review, an attempt to further clean up the samples was made by changing from liquid/liquid extraction after the protein precipitation to a solid phase extraction. Samples were precipitated as above, but the supernatant was collected and added to 5mL of water to reduce the % organic phase prior to addition to the column. Samples were mixed and loaded onto Waters Oasis HLB

Extraction Columns (3cc 60mg) (Waters, Milford, MA, USA) preconditioned with 2mL of methanol twice, 2mL of H<sub>2</sub>O, 2mL of 25/75 MeOH/H<sub>2</sub>O. The sample tube was rinsed with 2mL of water and the rinsing solution transferred to the SPE column. Each SPE column was washed with 3mL of 25/75 MeOH/H<sub>2</sub>O and 3mL of 50/50 MeOH/H<sub>2</sub>O. Samples were eluted from the column with 3mL of 90/10 ACN/H<sub>2</sub>O, dried down under nitrogen gas, and reconstituted with 50µL of ACN followed by the addition of 50µL of H<sub>2</sub>O. Samples were transferred to 250µL glass inserts, centrifuge at 4,000 rpm for 20 min, and analyzed by HPLC/MS/MS using our previously reported protocol with the following changes to reduce sample run time: linear gradient generated at 250µL/min: 5 min, 100% A to 100% B; 5-13 min, 100% B; 13-13.1 min, 100% B to 100% A; 13.1-20 min, re-equilibrate with 100% A with an injection volume of 20µL. The dwell time is now 50 milliseconds and optimum positive ESI conditions include: curtain gas, 40; collision gas, 10; ion spray voltage, 5500; and temperature, 500°C, ion source gas 1, 40; ion source gas 2, 35.

Processing of Volunteer and Patient Samples: Ten healthy volunteers, mean age 28 +/- 0.88 years and 15 patients scheduled to receive isotretinoin for severe acne (mean age 19 +/- 1.42) were recruited for assessment of serum retinoid levels as covered by this grant. These patients represent a subset of our ongoing study assessing the effects of 13cRA on skin levels of NGAL, sebum production, reduction in *P. acnes* counts and the effects of this drug on the phenotype of lymphocytes and monocytes. For this reason, the patient identification numbers begin at patient 18. Patient and volunteer samples were protein precipitated, solid phase extracted, resuspended, and run for HPLC/MS/MS as described above. Each run of patient samples included a standard curve using the above mentioned pooled pretreatment serum:RPMI matrix, spiked with 50ng/mL acitretin internal standard and 0, 0.3, 1, 2, 5, 10, 50, 100, 250ng/mL each of 13-*cis*, 9-*cis*, and ATRA. Spiked quality control samples (QCs) were run at 0.3, 1, 40, 125, and 200ng/mL.

#### Data Analysis and Results:

Eight-point calibration curves were constructed by plotting the peak area ratios of each of the three retinoids (13cRA, 9cRA, and ATRA) and the IS (Acitretin) versus the corresponding concentrations and fitting the data using linear regression with  $1/x^2$  weighting factor. Analyst software version 1.5.1 (AB Sciex) was used. Method accuracy and precision were evaluated using quality control (QC) samples prepared by spiking standard compounds at 4 concentration levels (0.3, 1, 40, 200ng/mL) into a pooled human serum sample for which the endogenous levels of the analytes were measured daily. Excellent linearity was obtained for the 8-point calibration curves over the concentration range of 0.3-250ng/mL for 13cRA, 9cRA and ATRA ( $r^2 > 0.99$ ). The method was found to be highly accurate with < 6.6% deviation from the nominal values and highly precise with inter-assay precision < 9.86%, and intra-day precision < 9.42% for all 3 retinoids.

Volunteer and patient baseline data were analyzed using an unpaired *t*-test (significance at  $p < 0.05$ ) and no significant differences were noted between the concentrations of the three retinoids (13cRA, 9cRA, and ATRA) (Figure 3) or the three 4-oxo metabolites of the 3 retinoids (Figure 4) found in normal volunteer samples and the acne patient samples at baseline.

A paired *t*-test was used to analyze patient sample baseline data with other time points in their treatment ( $p < 0.05$  is significant). Levels of all three retinoids and all three 4-oxo metabolites of

the retinoids were significantly increased in patients over baseline at all treatment time points (Figures 3, 4). Levels of 13cRA > ATRA > 9cRA, with ATRA levels approximately half of those of 13cRA and 9cRA levels much lower. There is, however, a great deal of intra- and inter-patient variability in the individual data (Figure 5, representative plot, similar data for 9cRA and ATRA, data not shown), which can be expected in a routine clinical setting.

Conclusions: With support of this grant we were successful in developing a new methodology to detect serum retinoid and metabolite levels using a solid phase extraction technique prior to HPLC analysis. This improved the standard curve and QC recovery as evidenced by the % relative standard deviation of < 6.6%. These data will be presented at the upcoming meeting of American Society of Mass Spectrometry and a manuscript will be prepared.

The large variation in serum retinoid concentrations was anticipated based on limitations of isotretinoin dosing in clinical practice. Since the medication is prescribed and obtained within a computerized registry system, it is not uncommon for patients to run out of medication prior to their visit. We have successfully shown in patient serum that 13cRA is isomerized to ATRA and to a lesser degree to 9cRA and were also able to detect the presence of the 4-oxo metabolites of all three retinoids. These data are supportive of our hypothesis that the induction of apoptosis by 13cRA coincides with the appearance of one or more metabolites/isomers of 13cRA, which has never been shown with patient samples.

Subject Type	Baseline/	1 week treatment	4 weeks treatment	8 weeks treatment	20 weeks treatment
V2	+	--	--	--	--
V4	+	--	--	--	--
V5	+	--	--	--	--
V6	+	--	--	--	--
V7	+	--	--	--	--
V8	+	--	--	--	--
V9	+	--	--	--	--
V10	+	--	--	--	--
V11	+	--	--	--	--
V12	+	--	--	--	--
P18	+	+	+	+	--
P19	+	+	+	+	--
P20	+	+	+	+	--
P21	+	--	--	--	--
P22	+	+	+	+	+
P23	+	+	+	+	--
P24	+	+	+	+	--
P25	+	+	+	+	+
P26	+	+	+	+	+
P27	+	+	+	+	+
P28	+	+	--	+	--
P29	+	+	+	+	+
P30	+	--	--	--	--
P31	+	+	--	--	--
P32	+	+	--	--	--

Table 1 Volunteer and patient demographics and samples processed. Volunteers (V) were individuals without acne and serum was only collected once as there was no treatment involved. Patients (P) were all individuals with acne who were scheduled by their dermatologist to begin treatment with 13cRA. Patient serum samples were collected prior to the start of treatment (baseline) as well as at 1, 4, 8, and 20 weeks after the start of treatment. For various reasons such as missing clinic visits or discontinuation of treatment, not all patients have complete serum sample sets that include all time points. A “+” indicates the presence of a sample and a “--” indicates no sample available.

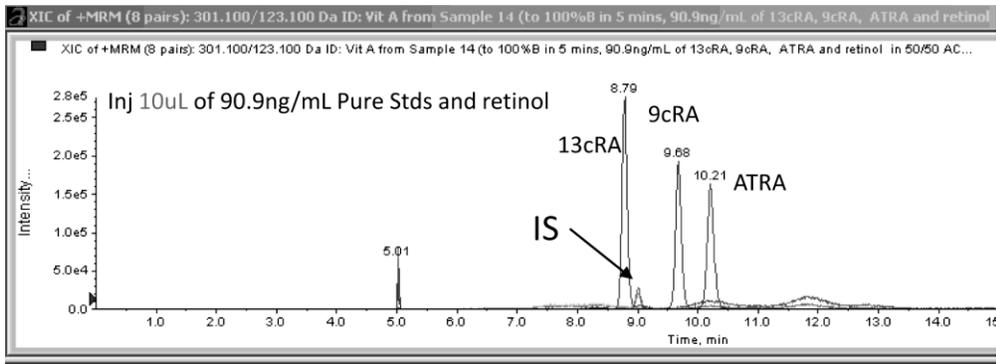


Figure 1 HPLC/MS/MS peaks for retinoids and retinol internal standard (IS). Retinol was detectable when pure standards were injected, but was difficult to detect after samples were precipitated and extracted and was, therefore, not an appropriate IS for our studies.

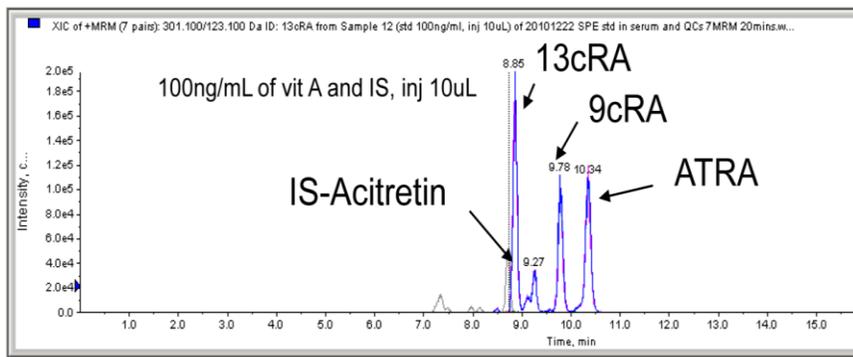


Figure 2 HPLC/MS/MS peaks for retinoids and acitreten. Acitreten was detectable when pure standards were injected as well as in protein precipitated and extracted samples and was, therefore, chosen as the internal standard for our studies.

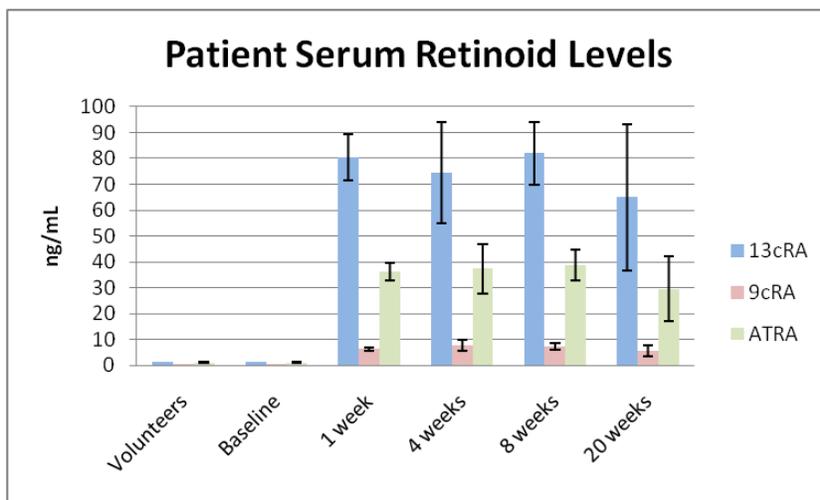
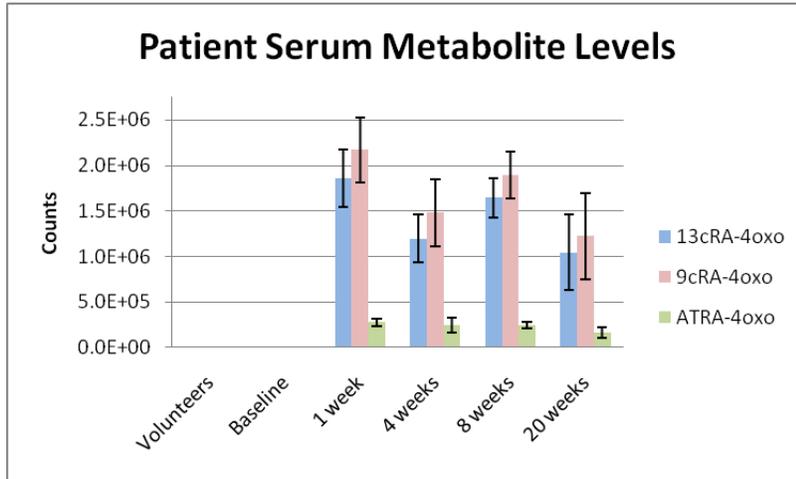
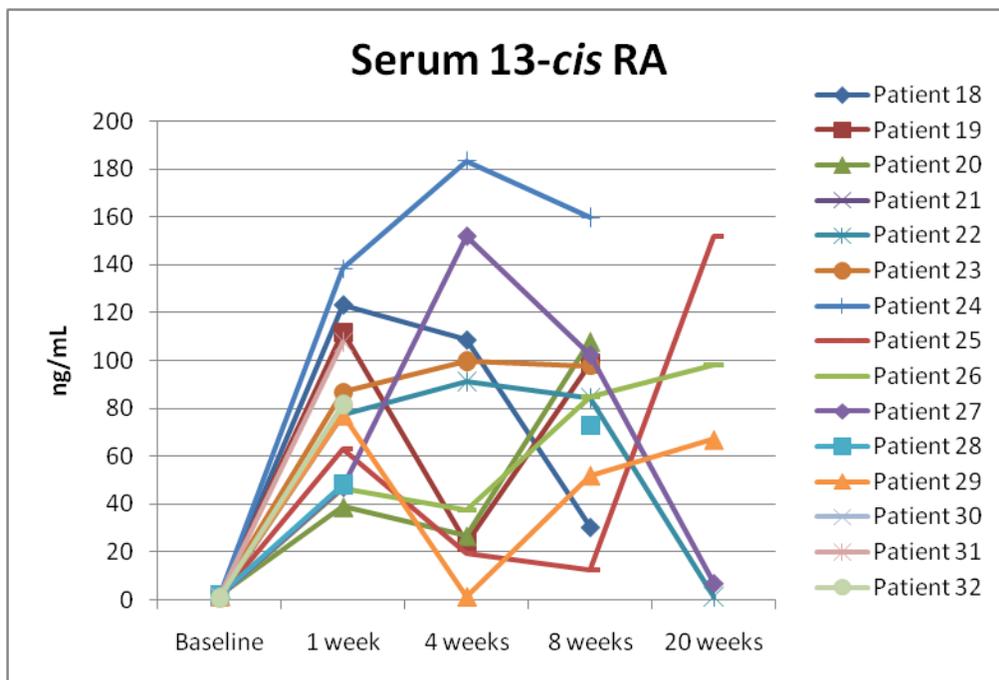


Figure 3 Patient serum retinoid concentrations. Samples were protein precipitated and solid phase extracted prior to HPLC/ MS/MS analysis. Acitreten IS (50ng/mL) was used to normalize samples and retinoid concentrations were calculated from a spiked retinoid standard curve (0, 0.3, 1, 2, 5, 10, 50, 100, 250ng/mL).



**Figure 4 Patient serum 4-oxo retinoid metabolite concentrations.** Samples were protein precipitated and solid phase extracted prior to HPLC/MS/MS analysis. Acitretin IS (50ng/mL) was used to normalize samples. No metabolite standards are commercially available; data can only be represented by raw counts. Relative levels of each individual metabolite can be compared but no comparisons can be made between different metabolites.



**Figure 5 13cRA concentrations in all patient samples at each time point.** Samples were protein precipitated and solid phase extracted prior to HPLC/MS/MS analysis. Retinoid concentrations were calculated by normalizing samples to spiked acitretin IS (50ng/mL) and concentrations calculated from a spiked retinoid standard curve (0, 0.3, 1, 2, 5, 10, 50, 100, 250ng/mL).

## **Research Project 30: Project Title and Purpose**

*Center for Computational Proteomics* - The purpose of this grant is to establish a Center for Computational Proteomics. The initial goals of this center are (i) to establish the infrastructure for the cutting edge computational tools we are developing which are capable of simultaneous measures of protein structure, function, and evolutionary information and (ii) to unify a core of researchers committed to utilizing this infrastructure towards answering important scientific questions related to human health and disease. Through the further development of these technologies, we hope to make large steps towards problems facing the health communities including rational drug design, drug delivery, and bioengineering. These tools will be available to the Penn State community at large and hopefully, in the future, to the entire scientific community.

### **Duration of Project**

8/9/2007 – 12/31/2010

### **Project Overview**

The protein problem has remained unsolved despite decades of heroic efforts. In principle, one expects that protein sequence ought to determine structure, function, and evolutionary (SF&E) characteristics; while in reality, there still is no reliable method for predicting the native state structure of a protein or its function given only its primary amino acid sequence either experimentally or theoretically. In addition, evolutionary measurements are stymied when homologous proteins are highly divergent from one another. It is also well known that the number of putative protein sequences of any given length is enormous yet only a very few of these can be classified as proteins which fold rapidly and reproducibly and have useful function. Despite this fact, there seems to be an astonishing simplicity to the protein problem because the number of distinct native state folds is extremely limited.

In general, the protein problem occurs due to the inability of current algorithms to identify homology between highly divergent protein sequences with statistical confidence. Moreover, sequence alignment is generally unreliable for matching two sequences when the pairwise identity is less than a threshold figure of about 25%, and alignments with lower identity (i.e. in the “twilight zone”) are usually treated as random events. However, a small number of conserved residues (~8% identity) can coordinate the 3D fold and/or function of proteins, with large portions of these proteins comprising heteromorphic pairs (i.e. protein sequences that form different folds depending on their sequence environment). This is likely because the key amino acids responsible for coordinating the fold (“signal”) are preserved in evolutionarily related sequences, while less evolutionarily taxing amino acid substitutions (“noise”) result in dilution of the identity signal below the threshold required by search algorithms to detect homology between sequences.

The recent explosion in the availability of knowledge-bases and computational techniques for the analysis of complex data has created an unprecedented opportunity for teasing out invaluable information from protein sequences. Starting with a basic premise that protein sequence encodes

information about SF&E, we developed a unified framework for inferring SF&E from sequence information using a knowledge-based approach in which we measure the similarity between a query sequence and a set of biologically relevant profiles in an unbiased manner. Results from this Gestalt Domain Detection Algorithm-Basic Local Alignment Tool (GDDA-BLAST) provide phylogenetic profiles that have the capacity to model SF&E relationships of various proteins. We propose that sequence information present within the “twilight zone” of sequence similarity can provide key insight into SF&E relationships among distantly related and/or rapidly evolving proteins. We expect that the tools and resources generated from this project will be accessible and user-friendly to the bench scientist, thereby speeding the discovery process of other clinically-relevant research endeavors. This specific aims of this project are to evaluate and optimize the performance of GDDA-BLAST on the detection of structural, functional, and evolutionary homology.

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

Just as physicists strive to develop a TOE (theory of everything) which explains and unifies the physical laws of the universe, the life-scientist wishes to uncover the TOE as it relates to cellular systems. This can only be achieved with a quantitative platform that can deduce and relate protein structure, functional, and evolution in a comparative fashion. Were this perfected, proper analyses would start to uncover the underlying physical laws governing the emergent behavior of biological systems. These ideas are far-reaching; however, progress is not possible without the attempt.

The long-term implication of this project is the development of a unified framework for *high-resolution and simultaneous measurements of structure, function, and evolution*. Should this be possible: (i) functional and evolutionary measurements could quantitatively inform structural modeling to derive accurate atomic resolution protein structures, (ii) structural and functional measurements could inform evolutionary histories to derive accurate evolutionary rates, deep-branch relationships, and homologous spaces within each protein, and (iii) structural and evolutionary measures would inform as to the location of functionalities contained within any protein and the regulatory elements which control these functions. Armed with this

information, speeds at which diseases could be understood and pharmacophores/therapies developed to combat them would likely increase dramatically.

It is our intent that the project presented here will help to pave the way for future work aimed at decoding the proteomes of disease causing organisms such as Influenza H1N1, *P. falciparum* (malaria), and antibiotic-resistant bacteria. We envision that in the future the GDDA-BLAST could be used to create proteomic libraries, which contain structural, functional, and evolutionary information. This innovation will likely provide unprecedented predictive power for a broad range of important questions such as those related to rational drug development, protein structure determination, cell biology, epidemiology, and bio-engineering.

### **Summary of Research Completed**

During this reporting period, the Center for Computational Proteomics (CCP) was actively working with experimentalists to model transmembrane containing domains in ion channels, exchangers, and viral non-structural proteins. These aims required effort to generate/refine Fold Specific Libraries (FSL) of position specific scoring matrices (PSSMs). Moreover, in order to model the 3-dimensional structure of a protein from primary amino acid sequence requires a rigorous computational protocol. While I do not have the space in this document to elaborate on all the projects related to integral lipid-binding domains, one project exemplifies the approach we are taking and how we are interfacing with experimentalists on campus: Han Q, Aligo J, Belton K, Chintapalli SV, Hong Y, Pattern RL, van Rossum DB, Konan KV. Conserved motifs in the transmembrane domains of NS4B protein are required for hepatitis C virus replication. In submission, *J. Virology*. (2011)

Hepatitis C virus (HCV) nonstructural 4B (NS4B) is an integral membrane protein, which plays an important role in the organization and function of HCV replication complex (RC). Although a lot is understood about its amphipathic N-terminal and C-terminal domains, we know very little regarding the role of the transmembrane domains (TMDs) in NS4B function. We hypothesized that in addition to anchoring NS4B into host membranes, the TMDs are engaged in intra- and intermolecular interactions required for NS4B structure/function. To test this hypothesis, we have engineered a chimeric JFH1 genome containing Con1 NS4B TMD region. The resulting virus titers were greatly reduced relative to JFH1 with further analysis indicating a defect in genome replication. We have mapped this incompatibility to NS4B TMD1 and TMD2 sequences and defined putative helix-helix dimerization motifs (GxxxG in TMD2 and TMD3; S/T cluster in TMD1) as key structural/functional determinants. Mutations in each of the putative motifs led to a significant decrease in JFH1 replication. Some mutations led to a disruption of NS4B foci implying that the TMDs play a role in HCV RC formation. Taken together, these data underscore the functional importance of NS4B TMDs in the HCV life cycle.

To visualize the spatial nature of these motifs and possible TMD interactions, we generated a 3-D homology model of TMD1-TMD4. Our biochemical data suggest complex interactions involving NS4B TMDs. To visualize the TMDs, we performed homology modeling to predict the conformation of JFH1 NS4B TMD region that has a similar or homologous amino acid sequence to known protein structures, in the Protein Databank (PDB) reference sequences. Homology modeling uses experimentally determined protein structures (templates) to predict the

conformation of another protein (target) that presents a similar or homologous amino acid sequence. This method generally consists of four major steps: (i) selection of the template protein(s) with known 3D structure and a similar sequence), (ii) target/template sequence alignment, (iii) building of the model by using MODELLER (a computer program which is used to construct 3D structures which automatically generates models) and (iv) selection of the final model based on the quality assessment of the resulting structures.

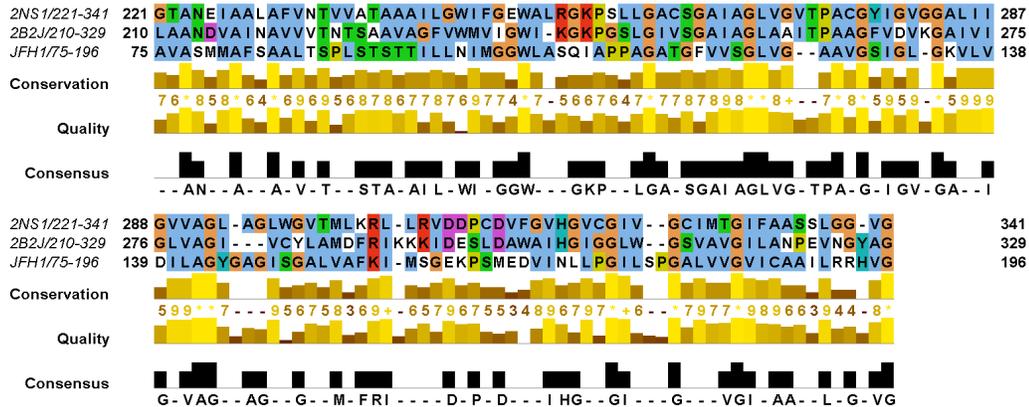
To select the appropriate structural template(s), we screened JFH1 NS4B amino acid sequence with Ada-BLAST (JIOMICS Hong et al. 2010 Manuscript in press; (<http://sing.ei.uvigo.es/ojs/index.php/jio/article/view/33/35>)). We ran NS4B against a library of PSSMs made from the structurally resolved Protein Databank (PDB) reference sequences (data not shown). After manual inspection of the alignment data, we isolated candidate templates which are: (i) membrane-bound, (ii) composed of transmembrane helices, (iii) glycine-rich (similarly to JFH1 NS4B), and (iv) aligned to JFH1 NS4B sequence (aa ~75-195) [see Fig. 1A]. These templates include two crystal structures: PDB code: 2B2J (an ammonium transporter Amt-1 from *A. fulgidus* and PDB code: 2NS1 (*E.coli* ammonia channel AMTB. Both Amt-1 and AmtB belong to the family of ammonium transport proteins (methylamine permeases/ammonium transporters), which are present in all domains of life. Each protein subunit comprises 11 TMD helices (TMD1-TMD11), with the N terminus located on the periplasmic side of the membrane and the C terminus on the cytoplasmic side. Each of the 11 TMD helices has different lengths ranging from 15 to 33 residues.

Using MUSCLE, we performed multiple sequence alignment of the templates and NS4B. The NS4B sequence (aa 75-195) was aligned to the structurally conserved regions of 2B2J (210-329) and 2NS1 (221-341) with a pairwise sequence identity ~19% and ~28%, respectively. We used these alignments to generate a set of 50 initial NS4B 3D models with the software MODELLER. Questions related to 3-D assessment are addressed by various methods in the MODELLER program. Perhaps the most straightforward is the molecular PDF (molpdf), which is the standard MODELLER scoring function, and is simply the sum of all restraints. In this score a low number is preferable. As such we rank ordered the 50 NS4B models and chose the one with the lowest molpdf score. This score is easily obtained as each PDB file is generated. While clearly not absolute measures, molpdf scores are a reasonable guide for assessment thresholds (see <http://salilab.org/modeller/tutorial/cryoem/assess.html>, for information on Modeller). The model with the lowest score, which was based on the *E.coli* ammonia channel AMTB structure, was chosen as the candidate structure. In addition to molpdf scores, one can assess a set of putative models based on stereochemistry although the function of MODELLER biases the output to more favorable scores, also reflecting on the quality of the respective template-target alignment. The distribution of phi and psi torsion angles, measured around all C- $\alpha$  atoms, is depicted by Ramachandran plots and represents another criteria of model robustness. We observe that within our top candidate NS4B monomer, 93.8% of the total torsion angles are in highly favorable regions (Fig 1b). Taken together, these quantitative statistics provide support that our model of the NS4B monomer is energetically favorable and may be biologically meaningful. It is important to state that even a successful model that fits with the experimental findings cannot fully represent the dynamic nature of an actual protein constrained in a highly complex lipid environment. Nevertheless, in general, homology models can provide useful guidance for visualization purposes and for generation of testable hypotheses.

We performed a quick optimization method on NS4B structure by moving all the side chains slightly to eliminate the possible small van der Waal's clashes. This is achieved by using online web server (FoldX: A force field for energy calculations and protein design. [www.foldx.crg.es](http://www.foldx.crg.es)). Here the FoldX program identifies all those residues which have bad torsion angles or van der Waal's clashes, or total energy, and we repair them. At first, it operates by looking for all the Asn, Gln and His residues and flips them by 180 degrees. This is mainly done to prevent incorrect rotamer assignment in the structure due to the fact that the electron density of D and E carboxamide groups is almost symmetrical and the correct placement can only be discerned by calculating the interactions with the surrounding atoms. In the second step, we do a small optimization of the side chains to eliminate small van der Waal's clashes by preventing the movement of the side chains. In the final step, we identify the residues that have very bad energies and we mutate them and their neighbors to themselves exploring different rotamer combinations to find new energy minima.

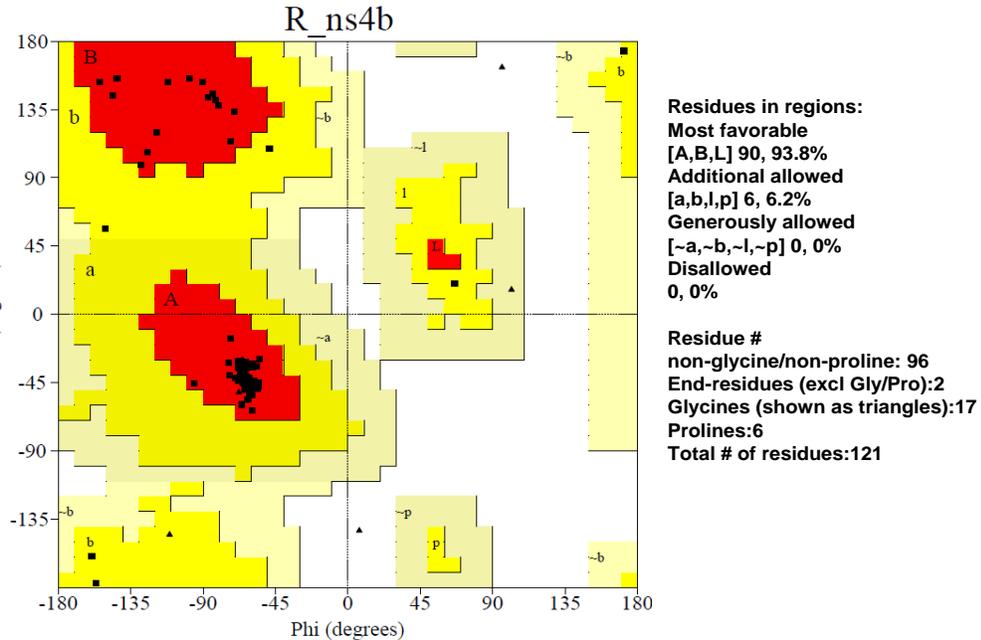
Fig 2 depicts different views of the final energy minimized JFH1 model of NS4B sequence (aa 75-195) including TMD1-TMD4. In panel A, the cytosolic and luminal sides of the membranes are defined by a dotted arrow whose position is informed: (i) by the known topology and membrane spanning region of the structural templates, (ii) by the solvent accessibility of the Walker A motif, which interacts with polar nucleotides and, (iii) by the position of a putative interfacial tryptophan (W104, TMD1), which resides just at the interface of the membrane and the ER lumen. In membrane spanning proteins, tryptophan residues are known to be located at interfaces between solvents and lipids. Each TMD is represented by secondary structure (TMD1, Blue; TMD2, Cyan; TMD3, Green; TMD4, Orange) and labeled accordingly. Overall, the JFH1 NS4B model accords surprisingly well with the predicted TMD boundaries used in the cloning of chimeric NS4B sequences and by TMHMM, a gold-standard Markov model for predicting transmembrane helices (2D). Based on the model and the biochemical interrogation of NS4B, we speculate that intramolecular interactions between TMDs could be biologically important for regulating the solvent accessibility of the Walker A motif. In the current model the binding pocket is obscured, partially by the van der Waal radii of TMD1 and TMD4 amino acids. Three types of conformational changes could open up the binding pocket: (i) force could be applied vertically with respect to the plane as the membrane (i.e. TMDs could stretch), (ii) force could be applied on the ER side of the membrane (Fig 2B, arrow), which like a clothespin would bring the TMDs closer together and potentially open the Walker A site, and/or (iii) force could be applied horizontally with respect to the plane of the membrane (Fig 2C, arrows) via the amphipathic N- and C-termini.

A

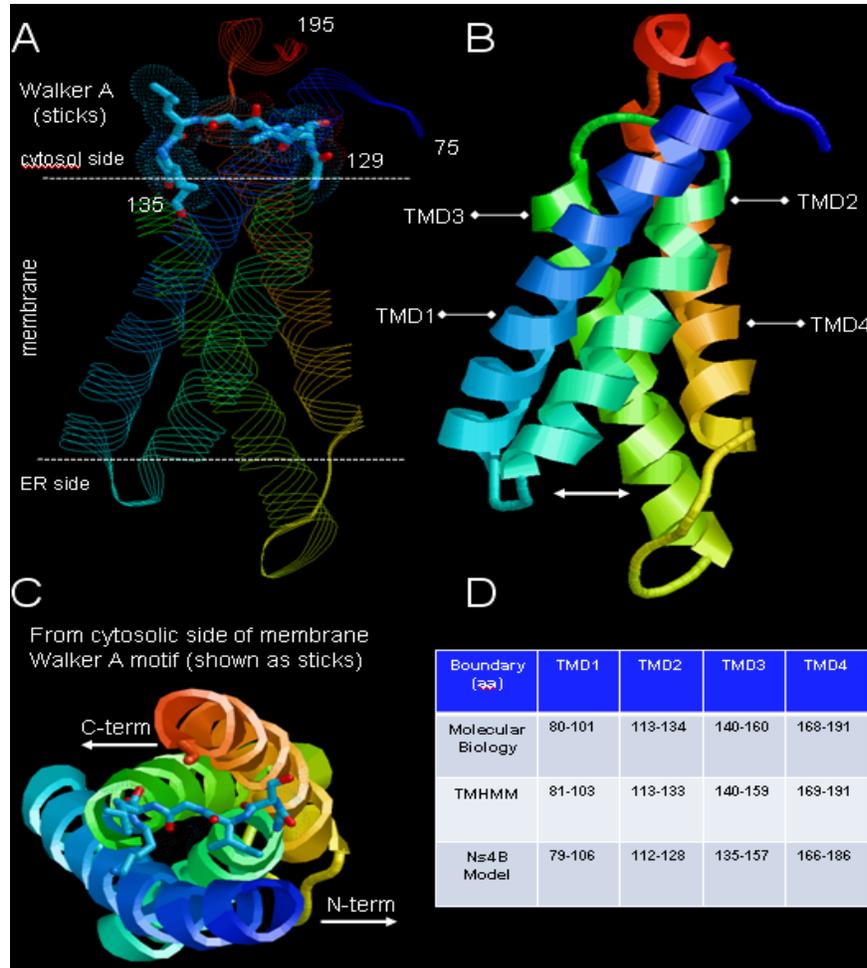


B

### Ramachandran Plot



**Fig 1: Template Alignment and Ramachandran plot of the NS4B homology model.** A. Multiple sequence alignment of JFH1 with ammonium transporters Amt-1 from *A. fulgidus* (2B2J) and AMTB from *E. coli* (2NS1), performed by alignment with MUSCLE. The pairwise percentage identity between sequences is as follows: 2NS1:2B2J (36.4%), 2NS1:JFH1 (28.0%), and 2B2J: JFH1 (19.7%). B. Ramchandan plot of the model NS4B. Out of the total 121 residues comprising the putative transmembrane region, 93.8% of the residues are in most favoured regions and 6.2% of the residues were in additional allowed regions. Based on an analysis of 118 structures (Resolution of at least 2.0 ang and R-factor no greater than 20%), Procheck expects a good quality model to have over 90% of amino acids in the most favorable regions.



**Fig 2: Homology Model of NS4B TMD1-TMD4.** A. Homology Model of NS4B TMD1-TMD4 sequence viewed from the side. The cytosolic and luminal sides of the membranes are defined by a dotted arrow. Here NS4B is shown in lines and the Walker A region is shown as sticks in the cytosolic side. Dotted lines reflect putative membrane boundaries. B. Cartoon depiction of the NS4B showing the four transmembranes. C. View of the NS4B monomer from the cytosolic face of the membrane. D. This table depicts the boundaries of the four TMDs proposed by different methods. Molecular Biology refers to those protein boundaries utilized to clone chimeric JFH1/Con1 viral proteins. TMHMM refers to a Hidden Markov Model, which is a gold-standard algorithm for defining transmembrane helices. NS4B model reflects the putative boundaries based on the structural model

### **Research Project 31: Project Title and Purpose**

*Structures of Protein in a DNA Replisome* - The crystal structures of key proteins, the primase and the clamp loader protein will be sought. Although the structures of several individual protein components are known, structures for several key proteins are not available. Since it is highly improbable that the crystal structure of an intact replisome will be solved, the structures of individual proteins are required to construct and infer the structure of the replisome. Our understanding of the function of the replisome is incomplete without its structure. In addition,

the tobacco funds will partially match a recently approved NIH-NCRR grant of \$500,000 for procuring a Rigaku Micromax 007 X-ray generator and Varimax optics, CCD detector and Xstream 2000 cooling system for the X-ray crystallography facility.

### **Duration of Project**

8/9/2007 – 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>.

### **Research Project 32: Project Title and Purpose**

*Galaxy Workbench for Analysis of Biomedical Data: Application to Overlapping Coding Regions* - Biomedical research is rapidly becoming extremely data intensive. High throughput experimental techniques are putting biology on par with the physical sciences in terms of raw data generating capacity. In this project we aim to capitalize on these developments. *First*, we propose to build on our success to date and develop novel algorithms for the two most rapidly evolving areas of biomedical research: (a) human variation analysis and (b) processing of data generated by next generation sequencing technology. *Next*, we will use the developed methodology for empirical and theoretical investigation of human genes with overlapping coding regions. This study will include *Ink4a* gene – a tumor suppressor locus implicated in ~50% of human cancers. Understanding the molecular dynamics of these genes will widen our understanding of human genome organization.

### **Duration of Project**

7/1/2008 – 6/30/2010

### **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 33: Project Title and Purpose**

*Using a Highmark-Medicare-Registry Merged File to Study Care, Outcomes, and Costs for Cancer* - The overall purpose of this project is to demonstrate how we can use existing linked cancer insurance claims and cancer registry databases to develop much more powerful and specific research regarding cancer care. This will allow us to link patient information (stage at diagnosis, age, co-morbidities, gender, etc.) with treatment information (surgery, radiation, chemotherapy, hormones, etc.) for a large population that will include both insured people under

65 years old and cancer patients who are Medicare recipients 65 years old and over. Such a strategy will allow us to investigate the factors that contribute to better quality cancer care.

### **Duration of Project**

7/1/2008 – 12/31/2010

### **Project Overview**

A data set linking Highmark insurance claims data with PA cancer registry data will be used to examine treatment patterns, outcomes, and costs for breast and colorectal cancers in Pennsylvania. We expect to include 50,000-100,000 patients with breast cancer or colon cancer insured by either Highmark commercial or the Medicare Advantage products in Pennsylvania in 1998-2006. The proposed method of looking at cancer data eventually can be applied to other cancer sites. There are two specific aims: (1) To develop an innovative and detailed method of linking cancer data from claims and registry data. Highmark has administrative claim data from both commercial and Medicare Advantage members. The new (combined) data set will be constructed by linking patient records from the Highmark claims data, their Medicare claims data, and the cancer registry data sets, to create an analytical file. It is important to note that this file will be stripped of all patient identifiers; and (2) To use this linked data set to study the relationship between various patient characteristics and treatment patterns for breast and colorectal cancer, the relationship between various treatment patterns for these cancers and costs and outcomes, the relationship between evidence-based research/recommendations from professional groups and actual practice patterns for these types of cancer, the effect of type of insurance on treatment patterns, and the effect of rural vs. urban patient locations on stage at diagnosis on treatment choices for these types of cancers.

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### **Other Participating Researchers**

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Tareq (Fabian) Camacho, MS, MA – employed by the Pennsylvania State University  
Patricia Gladowski, RN – employed by Highmark, Harrisburg, Pennsylvania

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

Because it is very expensive to gather detailed clinical data on the cancer care received by individual patients, it is important to develop innovative and valid methods to make use of existing claims and registry databases. Claims/registry databases can be used to discover

relationships between various processes of cancer treatment, subsequent outcomes of care, and the costs of different treatment options. While clinical trials give us information about the effectiveness of specific treatments for a very limited population of cancer patients (only those who agree and qualify for specific clinical trials), we must depend on other data to learn about process/outcome correlations for the various types of cancer patients who are actually treated, rather than those who are typically studied in clinical trials. We will use a linked data set to examine treatment patterns, outcomes, and costs for breast and colorectal cancers over a five year time period. We expect to include approximately 125,000 breast cancer patients and 55,000 colon cancer patients insured by either Highmark commercial or the Medicare Advantage products in Pennsylvania. The proposed method of looking at cancer data eventually can be applied to other cancer sites. This project includes both cancer control and health services research components. First, it is relevant to cancer control research because it investigates how information can be efficiently and effectively applied at the community level. Second, the project is applicable to health services research in that it examines the interface of the health care system with patients, and aims to improve outcomes and quality of care.

### **Summary of Research Completed**

During the past six months, we have continued to work on the last of our three specific studies that use the linkage database to explore the relationships between various patient/provider characteristics and treatment patterns, costs, outcomes as stated in our specific aim #2.

We focused on rectal cancer patients diagnosed in 2004-2006 for this particular study. Diagnosis years from 2004 onwards were used, since we intended to use the AJCC TNM (American Joint Committee on Cancer Tumor-Node-Metastasis) derived staging information to compare the actual treatment patterns to the treatment guidelines grouped by AJCC derived cancer stage. The AJCC derived stage information is available only since 2004 in our linkage data. We examined how the receipt of recommended treatment was associated with various patient and provider characteristics. In particular we looked at the hospital that performed the surgery, since rectal cancer surgery is technically demanding, and different providers may have a different quality of care. The hospital type studied included the size, the teaching status and the rural status.

It is largely unknown in the literature how the types of hospital which perform the rectal cancer surgery are associated with patient types, treatment patterns, costs and outcomes in rectal cancer. The hospital that performed rectal cancer related surgeries for the patient was considered to be the same as the reporting hospital in the cancer registry. The validity of this equivalence was verified. The hospital size was categorized into large vs. small depending on whether the number of hospital beds was at least 200. The teaching status of a hospital was determined from multiple publicly available data sources such as hospital profiles from the American Hospital Association.

To ensure high data quality, we cross-checked data from Highmark and the Pennsylvania Cancer Registry (PCR). Patients who had surgery as indicated in just one data source and not in the other data source were excluded. A total of 514 rectal cancer patients who had surgery as shown in both Highmark and PCR data were included in this specific study.

As to the relationship between hospital types and patient cancer stage, there was no statistical

difference in patient cancer stage between teaching vs. non-teaching hospitals ( $p=0.31$ ), nor between large vs. small hospitals ( $p=0.22$ ), nor between rural vs. urban hospitals ( $p=0.52$ ). However, patients with more severe co-morbid conditions tended to go to teaching hospitals ( $p=0.01$ ) for surgery, or/and urban hospitals ( $p=0.03$ ), but not necessarily large hospitals ( $p=0.30$ ).

It is recommended practice to have preoperative radiotherapy (RT) for stage II and III rectal cancer patients in order to reduce the rate of local recurrence. Interestingly, we found that teaching hospitals were associated with a much higher rate of preoperative RT treatment (57% vs. 28%,  $p<0.0001$ ) compared to non-teaching hospitals, despite the fact that similar stage rectal cancer patients were treated in teaching and non-teaching hospitals. This may indicate that teaching hospitals followed recommended treatment guidelines more closely than non-teaching hospitals. The rate of preoperative RT was not statistically different between large vs. small hospitals ( $p=0.13$ ). Though the preoperative RT rate was marginally significantly different between urban and rural hospitals ( $p=0.046$ ) in un-adjusted analysis, the urban/rural status became non-significant ( $p=0.20$ ) after controlling for other variables. In a multivariate analysis that accounted for other variables, teaching status was the only significant factor ( $p=0.002$ ) that predicted the receipt of preoperative radiation. This effect was independent of patient's age, comorbidity, cancer stage, gender, race and Medicare Advantage status.

After rectal cancer surgery, stage II and stage III rectal cancer patients may not necessarily need postoperative RT, as long as preoperative RT was received. Regarding postoperative RT for stage II and stage III rectal cancer patients, teaching hospitals had a much lower rate than non-teaching hospitals (13% vs. 30%,  $p<0.01$ ). Logistic regression showed that teaching hospitals had significantly ( $p=0.001$ ) lower likelihood of having postoperative radiation for stage II and III rectal cancer patients, after controlling for various patient and provider characteristics. This may suggest that teaching hospitals may be better at coordinating preoperative and postoperative treatment to follow recommended guidelines for rectal cancer.

Among rectal cancer patients of all stages, the mean surgery costs were not significantly different between teaching vs. non-teaching hospitals (\$1,446 vs. \$1,521,  $p=0.38$ ), nor between large vs. small hospitals (\$1,487 vs. \$1,529,  $p=0.70$ ), nor between urban vs. rural hospitals (\$1,489 vs. \$1,533,  $p=0.72$ ). After controlling for all other variables, hospital types remained to be non-significant factors for surgery costs.

We also studied the mean cancer treatment costs during the first 12 months after cancer diagnosis. Among all the rectal cancer patients, there was no significant difference between urban vs. rural hospitals (\$49,454 vs. \$41,705,  $p=0.18$ ), nor between large vs. small hospitals: (\$49,760 vs. \$42,411,  $p=0.15$ ). However, the mean cancer treatment costs were statistically different between teaching vs. non-teaching hospitals (\$55,031 vs. \$44,767,  $p=0.02$ ). In multivariate analysis adjusting for other factors, mean cancer treatment costs were significantly higher if patients had chemotherapy, co-morbid conditions ( $p=0.01$ ), were female ( $p=0.01$ ), or had a more advanced cancer stage ( $p<0.0001$ ). Age, gender, race, Medicare Advantage, receipt of radiation, and hospital characteristics including teaching status ( $p=0.19$ ) were not significant. Average chemotherapy costs were eight times as much as surgery costs and three times as much as radiation costs, resulting in the biggest component of rectal cancer treatment costs.

Survival analysis showed that teaching status was a significant factor for better survival, though teaching status did not affect surgery costs or cancer treatment costs as shown above. Using Cox proportional hazard model, teaching hospitals had significantly better survival (HR=0.39, p=0.008) than non-teaching hospitals after controlling for other variables. Fewer comorbid conditions and earlier cancer stage were also associated with significantly better survival; whereas gender, age group, race, rural/urban status, Medicare Advantage, hospital size and hospital rural status did not significantly affect cancer-specific survival. Teaching hospitals had significantly better survival, probably because teaching hospitals followed recommended guidelines more closely, and better coordinated pre-operative and post-operative treatment.

Apart from the above rectal cancer study, during this report period, we also reworked the data analysis on our previously reported overall studies using the updated final linkage data, and updated the study results accordingly.

### **Research Project 34: Project Title and Purpose**

*Structural Determinants of Closed State Inactivation of N-type Calcium Channels* - The enhancement of inactivation is an excellent mechanism to reduce calcium channel activity that is utilized by a class of antihypertensives called dihydropyridines (e.g. Norvasc®). The recent FDA approval of Prialit® for the treatment of neuropathic pain demonstrated the utility of blocking N-type calcium channels as a treatment for this debilitating human disease. However, the poor side effect profile of Prialit has limited its usefulness. Drawing from lessons learned from dihydropyridines, we are investigating drugs that enhance inactivation of these pain-mediating calcium channels. To facilitate this effort, we will use ion channel mutagenesis to determine structures within the N-type calcium channel that regulate inactivation. These structures would then be targeted for drug development to obtain lead compounds for further testing as pain treatments.

### **Duration of Project**

7/1/2008 – 6/30/2010

### **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 35: Project Title and Purpose**

*The Exaggerated Exercise Pressor Reflex in Rats with Heart Failure* - The project will attempt to determine the role played by mechanoreceptors and metaboreceptors in evoking the exaggerated sympathoexcitation seen both at rest and during exercise in patients with heart failure. These sympathoexcitatory responses are important because they affect blood flow in peripheral circulation resulting in altered (poor) muscle perfusion. In addition, these responses may be in part responsible for the exercise intolerance associated with heart failure. Resolving

the exact mechanisms of exaggerated sympathoexcitation is imperative in developing adequate treatments to improve physiological changes and the general quality of life for heart failure patients.

### **Duration of Project**

7/1/2008 – 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>.

### **Research Project 36: Project Title and Purpose**

*Epigenetic Chromatin Factors Involved in Cardiomyocyte Hypertrophy* - The purpose of this project is to understand the role of epigenetic heterochromatin factors in cardiac hypertrophy, a heart muscle disorder leading to heart failure and increased mortality. Several epigenetic heterochromatin factors: histone methyltransferase Suv39H1, histone H3(K9) methylation, and histone H2A.Z have been previously shown to control muscle cell differentiation and/or cardiac hypertrophy. Here we propose to examine chromatin organization in primary control and hypertrophic rat cardiomyocytes in parallel with cardiac myoblast cell culture and thus test the validity of primary cardiomyocytes and cultured cardiac H9C2 myoblast cell line as models for heterochromatin transitions. We also plan to conduct proteomic experiments with chromatin isolated from hypertrophic cardiomyocytes to reveal hypertrophy-associated changes of yet unknown epigenetic chromatin factors.

### **Duration of Project**

7/1/2008 – 6/30/2010

### **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 37: Project Title and Purpose**

*Blood-Brain Barrier Nutrient Transport* - The primary focus of this project is to determine the mechanism by which glucose in the circulation traverses the endothelial cells that make up the blood-brain barrier and thus gains access to the brain. Specifically we will investigate the regulation of the glucose transporter(s) in the luminal (blood facing) and abluminal (brain facing) membranes of the endothelial cells that facilitate the transport of glucose across the respective membranes.

**Duration of Project**

7/1/2008 – 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>.

**Research Project 38: Project Title and Purpose**

*Interaction between Survivin and Aurora B in Primary T Lymphocytes* - The major goal of this study is to determine the relationship of survivin and aurora B in primary T cells from survivin transgenic mice. We will define proliferation, expansion, cell cycle progression, cytokine production, survival, and memory development of T cells from survivin transgenic mice. Since survivin and aurora B have been suggested to promote T-cell proliferation and expansion, and sustain survival, we hypothesize that T cells from survivin transgenic mice will proliferate/expand strongly, and survive longer than T cells from normal background C57BL/6 mice.

**Duration of Project**

7/1/2008 – 6/30/2010

**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>.