Temple University

Annual Progress Report: 2010 Formula Grant

Reporting Period

January 1, 2011 – June 30, 2011

Formula Grant Overview

Temple University received $2,050,596 in formula funds for the grant award period January 1, 2011 through December 31, 2014. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

The Effects of Music Therapy Entrainment on Pain, Vital Signs, and Bowel Function of Cancer Patients - The purpose of this project is to gather preliminary data on the effectiveness of a specialized music therapy approach to pain management with cancer outpatients who have chronic pain. A cross-over design will be used to examine the effects of music therapy entrainment, a live music intervention based on principles of physics, on reported pain levels, vital signs, pain medication usage, and bowel function in 40 participants. Participants will receive one music therapy entrainment session and one sham treatment consisting of listening to pre-recorded music. In addition to measuring the above mentioned outcomes, three participants will be chosen at random to undergo MRI scans. The purpose of this is to assess brain activity while they listen to the recording of the music of their entrainment session as well as music of the sham treatment.

Anticipated Duration of Project

7/01/2011 – 6/30/2012

Project Overview

The overall objective of this project is to test the efficacy of a specialized non-pharmacological intervention, music therapy entrainment, on reported pain levels, vital signs, pain medication usage, and bowel function in cancer patients with chronic pain. Specific research questions are as follows:
1. Does music therapy entrainment significantly influence perceived pain, vital signs, breakthrough pain medication use and bowel functioning in cancer patients with chronic pain when compared to a sham music condition?
2. Which areas of the brain are activated by individualized entrainment music versus sham music?
3. What is the participant's experience of music therapy entrainment vs. a sham music condition?

This study will use a cross-over design with two treatment conditions: (a) one music therapy entrainment session and (b) one sham music listening session. Participants will be randomly assigned to treatment sequence A (music therapy entrainment followed by music listening) or treatment sequence B (music listening followed by music therapy entrainment). Perceived pain and vital signs will be measured before and after each session. Participants will also be asked to keep a pain diary to report daily pain scores, breakthrough pain medication usage, and bowel movements. Three participants will be randomly selected to undergo a MRI scan while they listen to the entrainment music and the sham music. The aim of the MRI scan is to assess potentially differential brain activity during these two music conditions. Finally, the participants will be interviewed regarding the qualitative aspects of their participation in the closing visit.

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Expected Research Outcomes and Benefits

It is expected that music therapy entrainment will result in reduced pain and enhanced vital signs in participants. It is also anticipated that the intervention may reduce usage of breakthrough pain medication. Reduced pain medication intake, in turn, may have a beneficial impact on the number of bowel movements. Medications are commonly used to treat chronic pain, however, even with medications for mild or moderate pain, such as nonsteroidal anti-inflammatory drugs, patients need to be monitored for potential side effects, including kidney, heart and blood vessel, stomach and intestinal problems. For more severe chronic pain, opioids are often prescribed; these have much more severe side effects, such as nausea, sleepiness, constipation, vomiting, difficulty in thinking clearly, and problems with breathing and sexual function along with potential overdose.

Music therapy offers potential advantages as treatment for pain because of low cost, ease of provision, safety, and lack of side-effects.

Finally, the MRI scans of participants will be the first to compare brain activity when listening to the recorded individualized entrainment music versus pre-recorded sham music.

Summary of Research Completed

This project will begin 07/01/11.

<u>Research Project 2: Project Title and Purpose</u>

Place-Based Interventions for Public Health: A Cross-Disciplinary Approach to the Study of Policing - The purpose of this study is: 1) to determine if the benefits of focused, foot patrol policing to reduce violent crime translates to a decrease in the incidence of drug-related illnesses (such as overdose) and physical assaults; and 2) to identify community variables that are associated with rates of crime-related illness or injury. Community variables include micro-places (e.g. alcohol outlets, parks) that serve as perpetual 'crime generators' as well as facilities (e.g. churches, health clinics) that contribute to the promotion of healthy behaviors. These data will provide evidence to support ways in which the police, along with city agencies and services can leverage their different tools and resources in a more targeted and collaborative manner to prevent crime and achieve shared health and safety outcomes.

Duration of Project

1/1/2011 – 06/30/2011

Project Overview

The objectives of the project are two-fold: (1) to provide pilot data regarding the associations between targeted, foot patrol policing (implemented in the spring and summer of 2009) and public health outcomes and; (2) to provide evidence regarding the factors that both negatively and positively relate to crime and crime-related illness and injury. These objectives translate into two specific aims:

Specific Aim 1: To compare the public health effectiveness of previous foot patrol interventions with control areas as measured by hospital Emergency Department (ED) visits for drug-related illnesses or violent injuries at Temple University, Episcopal and Northeastern Hospitals between January and September 2009 for Phase 1 foot beats and between April and September 2009 for Phase 2 foot beats.

We will collect data on physical trauma (assault, gun violence) and drug-related illness from Temple University Hospital, Episcopal Hospital and Northeastern Hospital in Philadelphia, and data on moderate to major trauma will be collected from the Pennsylvania Trauma Foundation. Databases will be queried for Emergency Department visits for physical assault, firearm inflicted injuries, stab or penetrating injuries and drug overdose. Patients' demographic data (e.g. age, gender) and street of residence for geo-coding will be collected.

These data will be related to primary crime outcomes as measured by incidence of criminal homicide, robberies and a majority of aggravated assaults - as well as secondary outcomes including drug incident detections (selling, buying, and possession of illegal narcotics).

Specific Aim 2: To identify and map community variables associated with rates of crime-related illness or injury as measured by the presence of both 'negative' and 'positive' micro-places.

This will involve a spatial analysis of micro-places that generate risky behaviors in selected parts of northern Philadelphia within the three service areas of the hospitals mentioned above and within the footbeat treatment areas; this will include bars and alcohol outlets, parks, and methadone clinics. We will also collect data on micro-places that are conducive to healthy behaviors, such as community health clinics, fresh grocers, police and fire departments, and schools from the City of Philadelphia Health Department and the Philadelphia Health Management Corporation. They will be geo-coded and then related to crime and injury data, including both Temple Emergency Department data and data from the 9-1-1 Emergency Medical Service (EMS) dispatch.

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Expected Research Outcomes and Benefits

A recent foot patrol intervention in the spring and summer of 2009 by the Philadelphia Police Department resulted in a 22% reduction in violent crime across 60 violent crime 'hotspots' (the 'treatment' areas) after twelve weeks in operation compared to control areas. Little is known, however, about the health effects of this policing intervention. Did violent crime reductions translate into improved health outcomes? By examining this question through various hospital and trauma data as well as spatial data, this study will provide insight into whether and how police can contribute to public health goals. Findings will benefit the city health department in its efforts to support the police in a shared commitment to reduce harm in the city.

The project will also provide a novel geographic perspective by mapping public areas where health outcomes are directly intertwined with crime. Such areas tend to be located in the proximity of noxious facilities like bars and city parks. Clearly articulating the spatial resolution and range of noxious facilities on both neighborhood crime and neighborhood health outcomes is a vital step in future policy determination and attempts to better manage unhealthy city spaces.

The project therefore has important implications for city policy and for the advancement of theories and methods for Epidemiological Criminology. A revised system of policing can

promote health areas and public health resources while working to deter high risk behaviors without alienating citizens who are seeking assistance with a range of health problems. We will share the findings of our study through a conference with the police department and city health department, a white paper, manuscript, and presentations at criminology and public health conferences.

Summary of Research Completed

Aim 1

Data

Admissions data were collected from Temple University Hospital (TUH) and the Episcopal Hospital (EH) in Philadelphia, which use the same medical record and data fields. As depicted in the map below (Figure 1), 7 footbeat areas and 7 control areas were located in a one mile radius surrounding EH, while 7 footbeat areas and 6 control areas were located in a one mile radius surrounding TUH.

The admissions data were obtained by screening the Temple University Health System patient registration database of the International Classification of Diseases (Ninth Revision, Clinical Modification (ICD-9-CM)) for principal discharge diagnosis codes (E960-E969) between March 1st and October 31st, 2009. These codes are used to identify "injuries inflicted by another person with intent to injure or kill, by any means". The sample identified 3187 unique visits to the Temple University Hospital Emergency Room, the Pediatric Emergency Room (same building) and the TUH Episcopal Campus Emergency Room. De-identified data from this sample was extracted into a Data Collection Form developed by the research team where additional demographic and medical information on each individual was collected. Patients' home addresses were used as a proxy measure for assault incident locations.

The time period of the data encapsulates a one-month pre-intervention period (March 1st to March 31st, 2009) and a one-month post-intervention period (September 27th to October 31st, 2009) for the foot patrol experiment. The data contains 2 intervention (peri) periods which reflect the staggered implementation of the foot patrols across the city. The Phase 1 period is April 1st to September 26th (approximately 5 months) and the Phase 2 period is July 7th to September 26th (Table 1).

Analysis

Patients' home addresses (N=3,187) were geocoded using *ArcGIS* (version 9.3). Excluding the addresses outside of Philadelphia and the ones listed as "unknown," a hit rate of 95% was achieved. *ArcMap* was used to calculate the number of home addresses in each of the following four areas: Phase 1 footbeat areas, Phase 1 control areas, Phase 2 footbeat areas, and Phase 2 control areas. In addition to the designated footbeat and control boundaries, 'buffer' areas were added to each of these areas.

After identifying the patients' home addresses that fell within each of the four areas, the date of the patient's admission into the hospital was used to determine the total number of patients admitted into the hospital for each day included in the study period. This process served to narrow down the original sample of 3,187 admissions to 1160 cases (N=549 for Phase 1 and N=

611 for Phase 2). The sample was then coded for footbeat intervention, footbeat buffer area, control area and control buffer area. (While analyses were initially run using all four groups, they were later collapsed into two areas – footbeat areas and control areas – as the numbers in each group were low and because the police officers reported that they traveled outside their given footbeat).

In Phase 1, there were 74 cases in the pre-intervention phase (40 in control and buffer areas and 34 in the footbeat and buffer areas), 271 cases in the intervention phase (162 in control and buffer areas and 109 in the footbeat and buffer areas) and 204 cases in the post-intervention phase (138 in control and buffer areas and 66 in the footbeat and buffer areas). In Phase 2, there were 308 cases in the pre-intervention phase (148 in control and buffer areas and 160 in the footbeat and buffer areas), 220 cases in the intervention phase (122 in control and buffer areas and 98 in the footbeat and buffer areas) and 83 cases in the post-intervention phase (43 in control and buffer areas and 40 in the footbeat and buffer areas) (Table 2). In addition, we also broke down the cases by month to better capture the variance over time (Table 3).

Due to a small number of occurrences (in some instances, zero), the team subsequently limited its sample to 3 categories of injury typically occurring in public spaces that were likely to have been influenced by foot patrol: (1) physical assault without a weapon; (2) stabbing or penetrating injuries; and (3) firearm-inflicted injuries.

Bivariate analyses were conducted within each Phase between the footbeat areas and the control areas first across the pre, peri and post intervention periods and then across each individual month between March (month 3) and October (month 10). The reason for the monthly analysis was due to the unequal time periods in each phase. (For the future, we are working on a way to standardize the measurement so that we have a rate of incidents/population. Given the small area of the footbeats – approximately 1.3 mi^2, it was difficult to use something like census track to create a standardized rate/population.) We also ran $\chi^2$to determine statistical significance, but results were inconclusive when run by month because there were several cells that had less than 5 cases.

Results

Phase 1 included a one month pre-intervention period, a 5 month intervention and a one month post-intervention period; Phase 2 included a 4 month pre-intervention period, a 3 month intervention and a 1 month post-intervention period. While we understood in advance that Phase 1 and Phase 2 were based in two geographically different areas, the intervention phase was longer in Phase 1 (n=5 months) than Phase 2 (n=3 months) thereby generating more intervention cases in Phase 1 than Phase 2. Due to the differences in the timing and number of months for the intervention period for Phase 1 versus Phase 2, we kept the analyses separated by phase.

In each phase, we present the data in two ways. First, we present the data summarized by pre-, peri and post intervention. However, given the issues noted above, summarizing the data this way "smoothes out" the data and does not provide detailed trends. Therefore, the second way that we present the data is on a monthly basis for the treatment areas and the control areas.

Phase 1
A visual inspection of trends in physical assault (without a weapon) over the pre-, peri, and post phases reveals a similar overall pattern in cases across treatment and control areas, but with a significantly smaller number of incidents in the treatment locations (Figure 2A). A month-by-month analysis provides a more nuanced picture showing the difference between reported cases of physical assault in the control areas versus the footbeat areas (Figure 2B).

While trends across treatment and control areas are similar during months 3 and 4 (March and April), assaults in the treatment areas develop a downward trend and then generally stabilize between months 7 and 10 (July and October), with minor upward and downward fluctuations. In contrast, physical assaults in the control areas follow an upward trend, with peaks at month 6 and month 8 (June and August). The data reveals that there could have been a short lag of time until the presence of foot patrols became known and their deterrent effects were achieved.

The physical assault (without a weapon) data proved to be more illustrative of potential trends than data limited to stabbing or penetrating injuries or firearm injuries. This is due largely to the smaller number of stabbings and firearm injuries that occurred overall. Below we show the same graphs as provided for physical assault by period and by month.

In the case of firearm injuries, we see that the number of cases in the control and treatment areas was very similar during the pre-intervention period (Figure 2C). Cases drop in the footbeat intervention group in month 4 and are mostly stabilized for the remainder of the intervention while cases in the control group fluctuate throughout the time period (Figure 2D). The low number of cases makes it difficult to truly assess differences.

In the case of stabbing injuries, again we see that the number of cases is low, but similar during the pre-intervention phase (Figure 2E). While the cases increased slightly during the start of the intervention and there was fluctuation through month 8 (August), the difference between footbeat and control areas continues through month 10 (October) (Figure 2F).

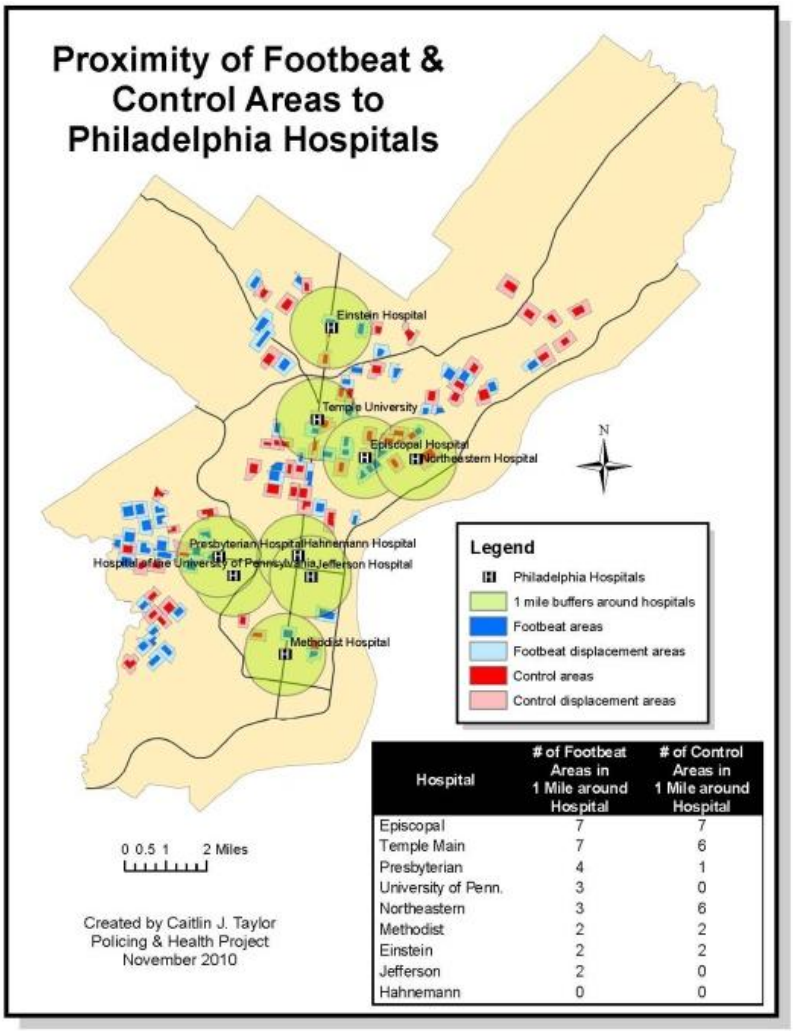
Phase 2
The findings in Phase 2 are not as clear as what we saw in Phase 1. A visual inspection of trends in physical assault (without a weapon) over the pre-, peri, and post phases reveals an overall downward trend in injury across treatment and control areas (Figure 3A). A month-by-month analysis provides a more detailed picture. There is considerable variation in the pre-intervention phase (months 3 through 6) and there is a greater divide between the control and intervention groups starting in month 8. By month 10 (post-intervention), it appears that the trend is equalizing between the two groups. The differences in the number of months in the pre, peri and post phases of the study is very important here and this explains the difference in the slope of the trend line in the figure broken out by pre, peri, post as opposed to broken out by month (Figure 3B).

Results are presented in relation to stabbing and penetrating injuries and firearm related injuries (Figures 3C through 3F), but due to the low number of cases, our discussion is limited to physical assault.

Aim 2

This aim has not been accomplished at this stage because it was discovered that the team will need to incorporate another data set maintained by the Philadelphia Fire Department - EMS data - which captures both the location of injuries throughout the city as well as hospital destination points (not limited to TUH and EH). These data will then need to be integrated with hospital data as well as police data on locations of related crimes.

Figure 1: Proximity of Footbeat and Control Areas to Philadelphia Hospitals



Hospital	# of Footbeat Areas in 1 Mile around Hospital	# of Control Areas in 1 Mile around Hospital
Episcopal	7	7
Temple Main	7	6
Presbyterian	4	1
University of Penn.	3	0
Northeastern	3	6
Methodist	2	2
Einstein	2	2
Jefferson	2	0
Hahnemann	0	0

Table 1: Study Phases

Study Phase	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Phase 1	O_{pre}	X	X	X	X	X	X	O_{post}
Phase 2	O_{pre}	O_{pre}	O_{pre}	O_{pre}	X	X	X	O_{post}
Note: O=observation, X=intervention								

Table 2: Case Breakdown of Each Phase for Pre, Peri and Post-Intervention for Control Areas and Footbeat Areas

Phase	Pre-Intervention		Intervention		Post-Intervention	
	Control Areas	FB Areas	Control Areas	FB Areas	Control Areas	FB Areas
Phase 1 (n=549)	40	34	162	109	138	66
Phase 2 (n=611)	148	160	122	98	43	40

Table 3: Breakdown of Cases by Month

Study Phase	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Phase 1 (n=549)	**O_{pre}**	**X**	**X**	**X**	**X**	**X**	**X**	**O_{post}**
Control Areas	40	37	37	42	37	50	44	53
FB Areas	34	31	27	25	20	23	26	23
TOTAL	74	68	64	67	57	73	70	76
Phase 2 (n=611)	**O_{pre}**	**O_{pre}**	**O_{pre}**	**O_{pre}**	**X**	**X**	**X**	**O_{post}**
Control Areas	30	37	30	42	39	49	44	42
FB Areas	33	43	47	28	39	39	32	37
TOTAL	63	80	77	70	78	88	76	79
Note: O=observation, X=intervention Cases include report of physical assault, sexual assault, child abuse, firearm injury, and stabbing injury								

Figure 2: TimePhase 1 Study Results.

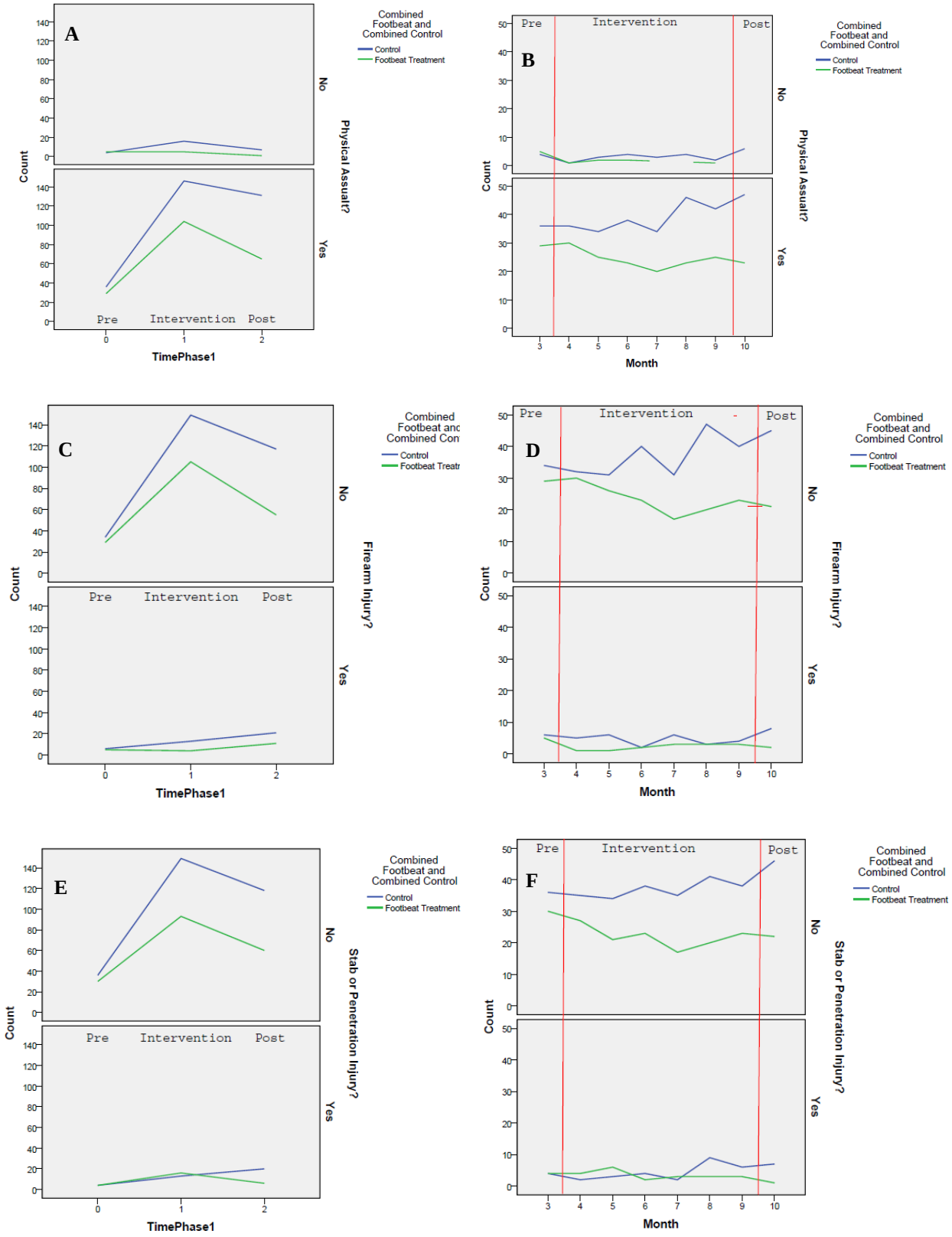
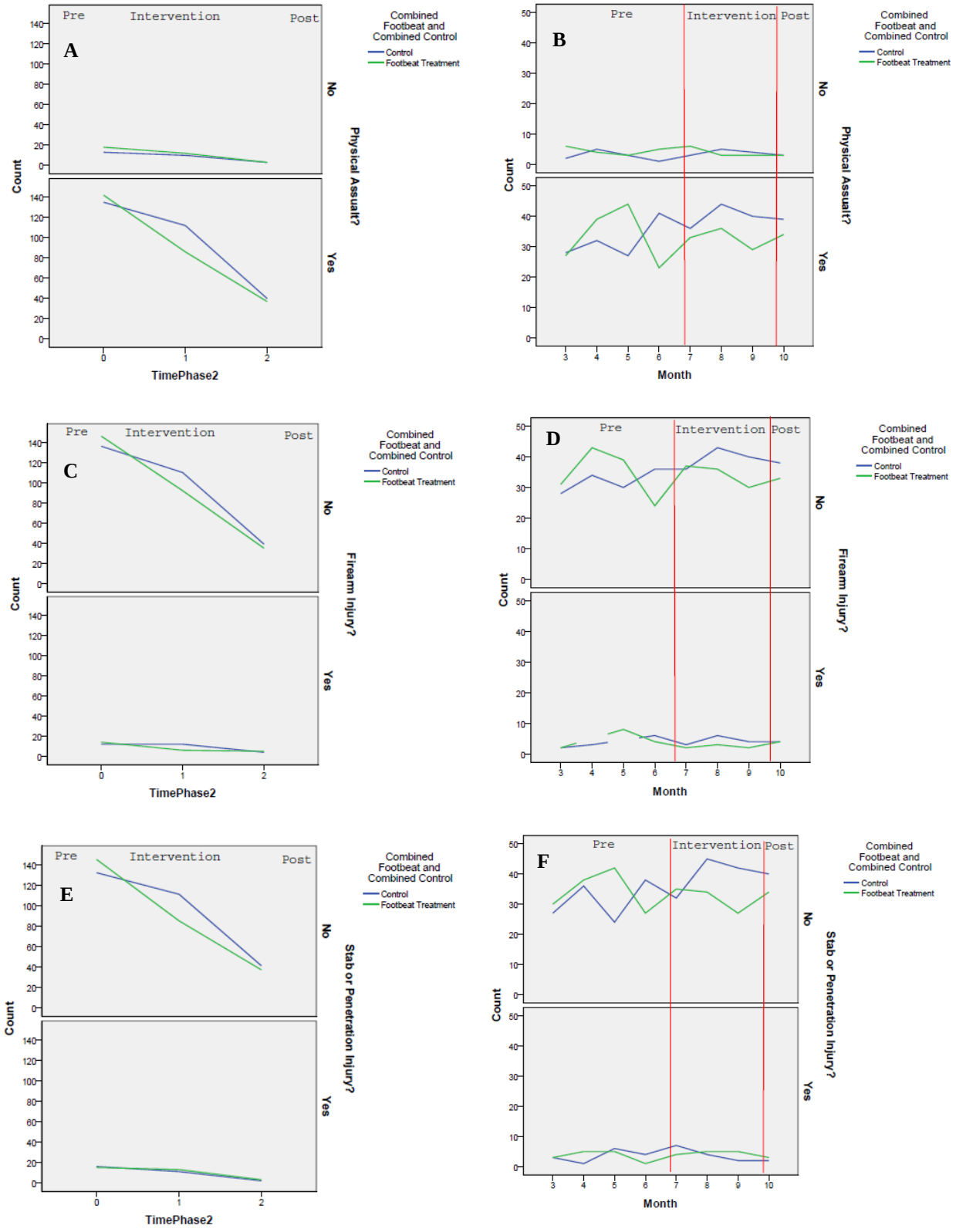


Figure 3: TimePhase 2 Study Results.



Research Project 3: Project Title and Purpose

Inflammation and Organ Tissue Damage - This project seeks to clarify the mechanisms by which alteration in the inflammatory response cause tissue damage and organ failure. Specifically, we will study critical steps of the inflammatory response to understand how: *1)* adhesion receptors expressed on endothelial cells and circulating leukocytes affect the immune response and subsequent organ damage, *2)* signal transduction processes initiated by such receptors regulate the inflammatory response, and *3)* cytokines produced by immune cells affect the overall outcome of the inflammatory response toward resolution or chronic inflammation. The overall goal is to uncover novel pharmacological strategies to prevent or treat the organ complications associated with chronic inflammation (e.g., neurodegenerative disorders, cardiovascular disease, obesity with insulin resistance, and physiological aging). These results will advance our understanding of the mechanism by which systemic inflammation increases organ morbidity and all-cause mortality in the population of the USA.

Anticipated Duration of Project

7/01/2011 – 12/31/2014

Project Overview

The overall goal of this project is to uncover novel mechanisms by which acute and chronic inflammation causes tissue damage and organ failure. Recently, basic and clinical research has highlighted a role for local or systemic inflammation in the organ tissue injury associated with acute (e.g., myocardial ischemia, viral and bacterial infections) and chronic (e.g., obesity, atherosclerosis, and Alzheimer) diseases states. The ability to mount an inflammatory response is essential for survival in the face of environmental pathogens and injury; however in some situations and diseases, the inflammatory response may be exaggerated and sustained without apparent benefit and even with severe adverse consequences. Understanding the mechanisms responsible for aberrant inflammatory responses in disease states is a necessary preliminary step to implement novel therapy. Accordingly, we seek to study three different, yet complimentary aspects of inflammation with the overall goal of understanding inflammation-mediated mechanisms of tissue damage using *in vitro* and *in vivo* experimental models that are relevant to human disease. Specifically, three specific aims have been developed to study: ***a)*** the endothelial expressed adhesive receptors that regulate infiltration of bone marrow derived inflammatory cells into organ tissues; ***b)*** the contribution to inflammation of Toll-like receptor (TLR) 2 receptor activation by curli amyloid fibrils; ***c)*** the modulation of the immune response by interleukin-4 in response to virus-induced inflammation.

The results of these studies will provide important information on the intercellular mechanisms of tissue inflammation in clinically relevant disease states. They will also provide new insight into the integrative mechanisms by which endothelial cells, circulating leukocytes, activation of Toll-like receptor, and cytokines synergize to deregulate the inflammatory response, thus producing aberrant immune responses leading to tissue damage.

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Expected Research Outcomes and Benefits

We expect to uncover novel mechanisms responsible for inflammatory induced tissue damage, a phenomenon that is common to several disease states (e.g., Alzheimer, obesity, cardiovascular disease, bacterial and viral infections, cancer and arthritis). In particular we anticipate finding that eCAMs, Toll-like receptor and selected cytokines play a key, mechanistic role in the fibrosis and remodeling associated with chronic inflammation. More importantly we will test whether that blockade of selected eCAMs, Toll-like receptor and changes in cytokine secretions prevents or attenuates tissue damage in inflammation. We will also define the molecular mechanisms responsible for deregulation and chronicity of the inflammatory response under our experimental conditions. Overall, this project will uncover novel mechanisms of inflammation that will provide a framework for developing new therapeutic strategies to avert vascular and organ damage in humans.

Summary of Research Completed

This project will begin 7/01/11.

Research Project 4: Project Title and Purpose

Inhibition of Leptin Receptor Signaling in Cellular Models of Rheumatoid Arthritis and Osteoarthritis - Osteoarthritis (OA) and rheumatoid arthritis (RA) are debilitating diseases whose progression can be promoted and/or aggravated by obesity. Leptin is a satiety factor regulating appetite and energy expenditure by acting on leptin receptors (ObR) in the hypothalamus. Leptin represents a critical link among nutritional status, metabolism and immunity. Recent evidence suggests a major role of the leptin / ObR system in the pathogenesis of OA and, perhaps, RA. Consequently, pharmacological downregulation of leptin activity could become a novel treatment for OA and RA. Our laboratories developed highly efficacious, specific and safe leptin-based peptide antagonists of ObR. This collaborative project will explore the applicability of our lead ObR antagonists in reversing characteristic OA and RA processes in vitro.

Anticipated Duration of Project

7/01/2011 – 12/31/2011

Project Overview

Several studies proved the role of leptin in the pathogenesis of autoimmune inflammatory conditions such as encephalomyelitis, type I diabetes, bowel inflammation as well as RA and OA. Leptin and its receptor were also shown to promote chondrocyte apoptosis, and consequent matrix synthesis impairment providing further evidence for their negative effects during secondary OA development. We developed short, pharmacologically stable peptide analogs of ObR-binding leptin site III that act as selective ObR inhibitors without any partial agonistic activity. The ObR antagonists reduce leptin-dependent growth and signaling in cancer cell lines at picomolar concentrations. In immunocompromised mice, low doses of the lead ObR antagonists suppress the growth of human breast cancer xenografts by 50% when administered either intraperitoneally or subcutaneously (sc). In an experimental rat RA model, the lead ObR antagonist administered sc reduces characteristic features of RA to baseline values.

This project aims to extend the studies to more serious inflammatory RA and trauma-induced OA. We will validate the ObR as a clinical target for RA and OA therapies and identify lead peptides or peptidomimetics for ensuing preclinical and clinical development. These goals will be accomplished by screening for inhibition of leptin-induced proinflammatory responses in chondrocytes and ObR-expressing immune cells. Special emphasis will be given to specificity to arthritic conditions and decoupling central nervous system and peripheral activities of the new ObR antagonists. We will look for peptides that can be eventually administered without the presence of attending physicians (eg, sc).

To this end, we will establish an ATDC5 cell- chondrocyte model in the laboratory, and will screen our existing ObR antagonists for leptin-induced chondrocyte responses and signaling. Later we will expand these studies to studying various immune cells. Then we will design and synthesize approximately 5 second generation ObR antagonists to decouple central nervous system and peripheral activities. After characterizing the in vitro stability and blood-brain barrier penetrating ability of the second generation leptin receptor antagonists we will repeat the above-listed studies in chondrocytes and immune-cells. The final selection of ObR antagonist optimized strong and selectively peripheral activity in the RA and OA cellular models will proceed for efficacy measurements in mouse models of adjuvant-induced and serum transfer RA.

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Expected Research Outcomes and Benefits

Musculoskeletal pain reached epidemic proportions in North America long time ago. Current treatment options are limited and the effectiveness of arthritis therapies remains insufficient. Non-steroidal anti-inflammatory drugs (NSAIDs) can be used to reduce acute inflammation, but they may not be efficient enough in controlling pain involving joints and nerve tissues because of difficulty to deliver them to the injury site. Corticosteroids that are given systemically can be useful in early stages of the disease but only as temporary adjunctive therapy. The most important recent advance has been the development of a group of biologic response modifier drugs, such as monoclonal antibodies (mAbs) targeting the tumor necrosis factor-α (TNF) or the CD-20 on B-cells. Since the introduction of TNF antagonists, the incidence of a lupus-like syndrome and serious skin reactions is dramatically increased. In addition, the treatment costs of anti-TNF mAb therapies are frequently prohibitively high and drug resistance can develop in long-time users. New biological targets are needed for OA and RA, and new chemical entities that are easy to mass produce are awaited. Compared with antibodies, the cost of peptide production is expected to be only a small fraction and the quality of the products could be easily controlled by pharmaceutically validated peptide synthetic processes. As such, ObR peptide antagonists would represent truly novel treatment options for musculoskeletal pain-inflicting diseases. The major innovative aspects of this project are:

- Extensive in vitro validation of ObR as a viable target to OA and RA;
- Development of unique, highly potent and safe peptide ObR antagonists for use as OA and RA treatment modalities (acting only in the periphery);
- Showing efficacy in a mouse model after subcutaneous (sc) administration mode.

Summary of Research Completed

This project will begin 7/01/11.

<u>Research Infrastructure Project 5: Project Title and Purpose</u>

Research Infrastructure Project: Multiphoton Imaging Facility – This project will fund a laboratory renovation to create a new Multiphoton Imaging Facility. Renovations will be made to existing space in the Department of Biochemistry located on the 6th floor of Kresge Hall. The updated facility will greatly improve capabilities for confocal microscopic imaging.

Anticipated Duration of Project

7/1/2011 – 6/30/2012

Project Overview

The purpose of the proposed infrastructure project is to renovate space and install the Multiphoton Imaging Facility on the 6th floor of the Kresge Building within the Department Biochemistry. Multiphoton microscopy is a powerful new tool that facilitates the study of discrete cellular processes within the three-dimensional, natural environment of intact organs in living animals. The major users will be a group of School of Medicine investigators drawn from a wide range of disciplines, including Biochemistry, Physiology, Pathology, Pharmacology, Anatomy and Cell Biology, Pulmonary Medicine, Cancer Research Institute and Rheumatology.

The renovated space will be centrally located in the School of Medicine to enable convenient access for all users, and will be maintained by the Biochemistry Department under the supervision of the PI and Microscope Advisory Committee. Investigators from diverse fields will have new opportunities for interaction and the initiation of interdisciplinary research projects, which will significantly advance their projects. Overall, the facility will provide a resource for noninvasive, microscopic medical detection and diagnosis of several diseases and provide the first state-of-the-art multiphoton imaging system to support the Temple University biomedical research community.

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Expected Research Outcomes and Benefits

The expected outcome of this project is to complete the required building modifications for a new multiphoton imaging facility and install the new multiphoton imaging equipment. The required infrastructure changes include complete renovation of the cooling capability for the floor to accommodate the extreme heat load produced by the new equipment. In addition, extensive plumbing, electrical, and data access modifications are required to allow operation of the facility. Completion of the renovations and installation of the imaging equipment will greatly increase our ability to examine cellular function in vivo, and expand our understanding of developmental, homeostatic, and pathologic processes. MP microscopy allows us to visualize dynamic processes for prolonged periods of time deep within intact organs with minimal toxicity, providing a convenient means to translate *in vitro* findings into *in vivo* settings. This

project's renovations will facilitate highly promising studies in a wide range of projects. The centrally located space will also offer the opportunity for multidisciplinary interactions between investigators and translational research groups. By combining novel molecular tools, innovative mouse models and MP microscopy, we expect to obtain new insights at the mechanistic level into the pathophysiology of disease.

Summary of Research Completed

This project will begin 7/01/2011.

Research Project 6: Project Title and Purpose

Immunomodulatory Cannabinoids as Potential Therapeutics for Transplant Graft Rejection – Compounds that bind to Cannabinoid Receptor 2 will be investigated for their potential to increase survival of skin and organ grafts. Compounds of this type should suppress immune reactions but not have psychotropic activity. The efficacy of these compounds in combination with standard anti-rejection therapy will also be investigated.

Anticipated Duration of Project

7/01/2011 – 12/31/2012

Project Overview

Current therapies for organ and skin transplantation have deficiencies, as long-term use results in unwanted side effects including nephrotoxicity, neurotoxicity, diabetes, and liver dysfunction. Synthetic cannabinoids that bind selectively to the cannabinoid receptor 2 (CB2) have been shown to have anti-inflammatory and immunosuppressive activity. Compounds of this class do not have the psychogenic effects of marijuana or its constituents because the CB2 receptor is expressed almost exclusively on cells of the immune system. The psychogenic effects of abused cannabinoids result from binding of ingredients in marijuana to the CB1 receptor, which is expressed on neurons. This anatomical separation of expression of CB2 receptors permits design of compounds that will affect the immune system but not the nervous system. This project seeks to assess the potential of CB2 binding compounds, alone or in combination with standard anti-rejection therapies, to depress graft rejection. There are two Aims. One will use an in vitro assay that is an accepted correlate of graft rejection, the Mixed Lymphocyte Reaction, to assess the immunosuppressive capacity of various compounds, alone and in combination with standard anti-rejection therapies. Aim 2 will test the potency of these compounds alone or in combination with standard anti-rejection therapies in vivo using mice with grafted tissues. The in vitro assays will be used as a screen for compounds and drug combinations. Those that are most effective in vitro will be tried in vivo.

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Expected Research Outcomes and Benefits

This research has the potential to develop a new class of compounds that bind selectively to Cannabinoid Receptor 2 (CB2), expressed on cells of the immune system, as effective therapies in prolonging skin and organ grafts. Based on preliminary in vitro data, CB2 selective compounds appear to have the ability to decrease reactivity of leukocytes for foreign tissue. CB2 active compounds were shown by us to decrease lymphocyte proliferation in response to foreign tissue and to decrease the secretion of proteins (cytokines) that activate cells of the immune system. These results were obtained using the Mixed Lymphocyte Reaction, an assay that is generally accepted as an in vitro correlate of graft rejection. This project will further evaluate the potential of these compounds for preventing graft rejection by taking the experiments in vivo. Mice receiving grafts will be treated with the CB2 active compounds and graft survival compared with controls and with those of mice receiving standard anti-graft treatment. In addition, combinations of reduced doses of standard therapies will be tested with addition of CB2 compounds. Combination therapies may reduce toxicity of currently available anti-rejection drugs. The new compounds may be effective by themselves, or may be useful in combination with reduced doses of currently accepted anti-rejection regimens, which may reduce the long-term toxicity of the current standard regimens.

Summary of Research Completed

This project will begin 7/01/2011.

Research Project 7: Project Title and Purpose

Differentiation Therapy for Leukemia – The purpose of the project is to evaluate the therapeutic efficacy of the peptide, angiocidin, for the treatment of leukemia. We propose to determine if angiocidin can block the engraftment of human leukemia cells in a mouse model of leukemia. We will evaluate the effect of angiocidin on the engraftment of at least three patient leukemia cells. These will be patients with acute myeloid leukemia.

Anticipated Duration of Project

1/1/2011 – 12/31/2011

Project Overview

This research project will have a direct and positive impact on leukemia cancer research since it proposes an entirely novel and unique strategy for improved treatment of leukemia cancer which can readily be applied for clinical evaluation. Acute myelogenous leukemia (AML) is a hematogenous malignancy in which the bone marrow makes abnormal myeloblasts that fail to differentiate into mature leukocytes. These myeloblasts lack immune function, are invasive and can quickly lead to mortality. Current treatment options involve chemotherapy and durable remissions are low requiring stem cell transplants to achieve a cure. We propose that recombinant angiocidin, a human protein that is easily expressed and purified from bacteria and previously shown to possess potent anti-tumor activity can be used to treat AML based on our novel discovery that angiocidin was able to differentiate a highly studied monocytic AML cell line (THP-1) into normal-like macrophage cells. In this project, we plan to evaluate the anti-tumor activity of angiocidin against three human AML primary cells in a mouse model of leukemia.

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Expected Research Outcomes and Benefits

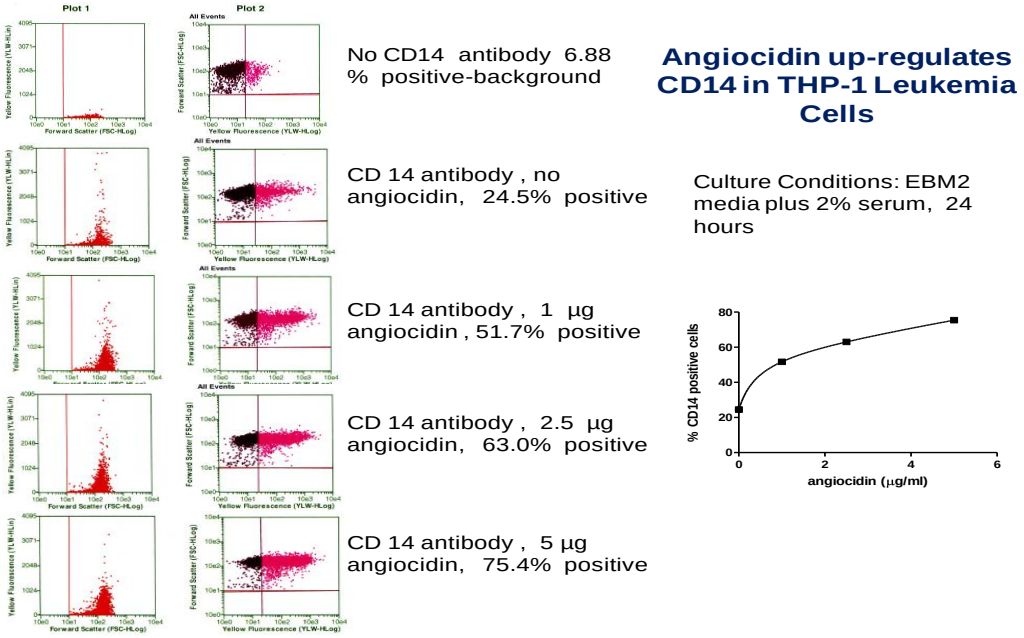
An estimated 245,225 people in the United States are living with, or are in remission from, leukemia. Approximately 21,870 deaths in the United States were attributed to leukemia in 2009. The most common types of leukemia in adults are acute myelogenous leukemia (AML), with approximately 12,810 new cases in 2009, and chronic lymphocytic leukemia (CLL), with about 15,490 new cases in 2010. In 2009, in the United States, leukemia was the fifth most common cause of cancer deaths in men and the seventh most common in women. AML accounts for 80% of all acute leukemia in adults. The trend in overall incidence is slowly increasing. In general, 60% to 80% of all treated AML patients achieve initial remission, but 5-year survival rate is only 15% in the adult population of AML and has been stably or slowly increasing. Standard AML therapy requires intensive combination chemotherapy which leads to significant treatment-related toxicity, especially in elderly patients. Therefore, new therapeutic options for the treatment of leukemia are urgently needed. Our therapeutic targets unique

proteins in the provisional tumor matrix and tumor surface such as α2β1 integrin, and previously published data indicate that systemically administered angiocidin homes to both the tumor and its vasculature and should be equally effective against the primary tumor as well as tumor metastases. Our new data provide evidence that angiocidin may have the potential to treat systemic circulating hematogenous tumors by differentiating them *non-toxically* into normal cells. Therefore, these therapeutics could provide an alternative non-toxic treatment choice for leukemia patients or could also be used in combination with currently approved cytostatic drugs for the treatment of leukemia.

Summary of Research Completed

We are in the process of developing the flow cytometric protocols for evaluating the expression of differentiation antigens induced by angiocidin and its two active site peptides FCTGIRVAHLALKHRQGKNH (20 mer) and ALKHR (5 mer). We are using the human leukemia cell line THP-1 to develop the protocols. We established the culture conditions using the growth media EBM2 media plus 2% serum and a 24 hour time frame to get maximal differentiation. Thus far we have evaluated the macrophage marker CD14 and found that angiocidin can up-regulate this marker 50-70% of the cells under our culture conditions as shown in Figure 1. We plan to investigate more makers such as CD54, HLA-DR, CD69, CD49b and EMR1 in the THP-1 cells as well as primary patient cells.

Figure 1. Angiocidin up-regulates the macrophage differentiation marker CD14 as measured by flow cytometry.



Research Project 8: Project Title and Purpose

Social Influences on Alcohol Consumption in Adolescent Versus Adult Mice – The presence of peers is associated with increased risk taking during adolescence, including alcohol and drug use, but the underlying neural mechanisms for this "peer effect" are not understood. Importantly, a similar effect is not seen among adults. Previous research by one of the present investigators indicates that the effect may operate via the impact of peer presence on the adolescent brain's reward processing system, such that the presence of peers increases the salience of other rewards and thereby biases individuals toward the potential benefits of a risky choice. This project will examine the feasibility of modeling this process in rodents, and will examine the differential impact of "peer" presence on alcohol consumption among juvenile and adult mice.

Anticipated Duration of Project

7/1/2011 – 6/30/2012

Project Overview

The presence of peers increases risky behavior among adolescents, but not adults. This "peer effect" is likely due to the impact of the presence of peers on reward processing and is mediated neurally by hyperactivation of brain regions known to be associated with reward sensitivity. Understanding the processes through which peers influence adolescent risk-taking has important implications for public health, since a preponderance of adolescent recklessness, including binge drinking, occurs in the presence of their friends. To date, experimental documentation of the peer effect has relied on simulated outcome variables that mimic real-world risky behavior. It is important to show that the presence of peers affects actual risky behavior, such as drinking alcohol, but legal and ethical issues preclude the conduct of experiments in which alcohol consumption is compared among teenagers when they are alone versus with their peers. However, this is a topic to which animal models can be readily applied, and lessons learned from such work can inform our understanding of human behavior and development.

All mammals experience a stage of development comparable to the biological stage in humans that is labeled adolescence. Importantly, the behavior of adolescent and adult rodents is quite different, as are their responses to alcohol. For example, social interaction among adolescent, but not adult, rats is facilitated in familiar environments by low doses of alcohol; whereas inhibition of social interaction is seen at moderate doses of alcohol in adults and only at high doses in adolescents. Adolescent and adult rodents also differ with respect to voluntary consumption of alcohol, with adolescent rats and mice consuming more alcohol than adult rats and mice. A key unanswered question is whether there is an interaction between social setting and alcohol consumption among rodents. The aim of the proposed research is to test the hypothesis that adolescent mice will consume more alcohol when the alcohol is available in a social setting than they will consume when they are alone. Being able to evaluate social behavior and alcohol consumption simultaneously in rodents will provide valuable novel information that will aid in understanding the factors involved in alcohol consumption in adolescent humans.

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Expected Research Outcomes and Benefits

The chief contributors to morbidity and mortality among adolescents are self-inflicted, and one of the most common threats to adolescent health and well-being is alcohol use. Adolescents typically engage in risky behavior (including drinking) when they are with their peers. Previous experimental research has demonstrated that effect of peers on adolescent risk taking is mediated through the impact of peer presence on the brain's reward processing system, but this "peer effect" only has been demonstrated experimentally using laboratory tasks that merely simulate real-world risk taking. It is important to know whether a comparable peer effect is seen when the outcome in question is a genuine, health-compromising risk behavior, such as drinking. Legal and ethical issues, however, preclude the possibility of studying peer effects on adolescent alcohol use experimentally. We believe that the peer effect on adolescent alcohol consumption can be profitably studied using animal models, which permit a more detailed examination of the underlying neural processes. In order to fully study this phenomenon, we must first demonstrate that we can create it experimentally in a controlled laboratory context. The expected outcome of this project therefore is the demonstration of the feasibility of studying peer influences on adolescent alcohol consumption using mice. The long-term benefit of this program of research is a fuller understanding of factors that influence alcohol use during adolescence and, ultimately, more effective preventive interventions to reduce alcohol use and abuse.

Summary of Research Completed

Project will begin 7/01/2011.

<u>Research Project 9: Project Title and Purpose</u>

The Role of G Protein-Coupled Receptor Associated Sorting Protein 1 (GASP-1) in Breast Cancer Progression – The purpose of the project will be to evaluate the prognostic significance of GASP-1 in breast cancer detection and diagnosis.

Anticipated Duration of Project

1/1/2011 – 12/31/2011

Project Overview

The "2-D High Performance Liquid Electrophoresis" (2-D HPLE) technology employing polyvinylidene fluoride (PVDF) membranes instead of polyacrylamide gels for the high resolution separation of protein complexes is a *totally novel* protein separation system. This new system possesses many advantages over the currently used 2D-SDS-PAGE procedure. Among the advantages are high resolution, reproducibility, sensitivity, speed and the capacity to detect protein-protein interactions under non-denaturing conditions. 2-D HPLE has the capacity to separate both hydrophobic and hydrophilic serum albumin complexes into distinct, well-resolved spots which cannot be accomplished by conventional 2D-SDS-PAGE. The separation of albumin complexes in 2-D HPLE is based on their net charge or isoelectric points (pI). The association of a newly produced cancer protein (or its fragments) with a pre-existing albumin complex changes its pI and this new complex will migrate to a different location on the PVDF membrane, allowing its detection among hundreds of already present albumin complexes. Therefore, 2-D HPLE technology can detect early "biochemical events" occurring in breast tissue that lead to breast cancer. To illustrate the potential utility of 2-D HPLE for detecting breast cancer, we have compared the serum albumin complex profiles of sera from 5 matched normal volunteers and 3 patients each with Early Stage 1, and Stages 1, 2, 3, 4 breast cancer (15 patients) and reproducibly found a series of new protein complexes appearing in the cancer sera but absent in normal sera. *The validity of our 2-D HPLE approach was confirmed by the identification of one cancer protein (biomarker) from Stage 1 sera that is over-expressed in tumor tissue with respect to adjacent normal tissue in all 7 cases of breast cancer patients.* This protein, G- protein coupled receptor-associated sorting protein 1 (GASP-1), has an important function in mediating the trafficking of G-protein coupled receptors and targeting them for degradation in lysosomes. In this project the expression of GASP-1 will be evaluated in a series of breast cancer tumor sections and its expression will be correlated with the severity of the disease.

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Expected Research Outcomes and Benefits

We anticipate that increased GASP-1 expression will correlate with worsening stage and histological grade. We additionally anticipate that hyperplastic lesions, especially from those patients that eventually develop carcinoma, will show increased GASP-1 expression. The health benefit is that a new breast cancer biomarker will be described that can be used for the early

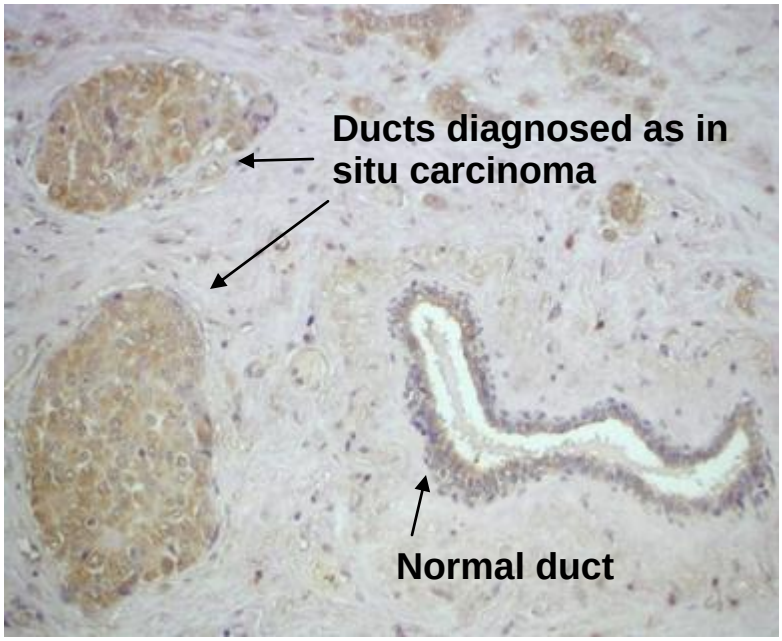
detection of cancer.

Summary of Research Completed

We are in the process of setting up our immunohistochemical staining techniques for evaluation of the expression of GASP-1 in breast cancer tumors. Our plan is to stain breast cancer tissue arrays and evaluate the expression of GASP-1 in these samples and determine if the level of expression correlates with the stage of the disease. The following is the protocol that we have developed and an example of a tumor section that contains a malignant duct diagnosed as in situ carcinoma and an adjacent normal duct (Fig. 1). We see strong staining of the carcinoma ductal cells and much less in the normal cells of the duct. From this experiment, we conclude that GASP-1 is expressed more highly in the ductal epithelium of tumor cells than in the ductal epithelium of normal ducts.

<u>Protocol for Immunohistochemical Staining.</u>
Breast Cancer microarray slides CBA4 and BR1503 will be purchased from IMGENEX, San Diego, CA and US Biomax, Inc, Rockville, MD, respectively. The slides will be deparaffinized in xylene, subsequently immersed in graded alcohols (100%, 90%, 70% ethanol) and rehydrated in dH2O. Sections will then be teated with citrate buffer at 95° C for heat-induced epitope retrieval. Samples will be washed with phosphate buffered saline (PBS) and blocked for endogenous peroxidase activity with 3% hydrogen peroxide in methanol for 20 min. After washing sections in PBS, samples will be incubated with 5% normal horse serum in PBS for 1 hour at room temperature and then they will be incubated with a 1:4000 dilution of rabbit anti-GASP-1 peptide in PBS at 4° C overnight. Slides will be washed in PBS and then will be incubated for 30 min with a biotinylated secondary antibody and finally incubated with the HRP-streptavidin complex for another 30 min as described in the Vector ABC staining kit (Vector Laboratories, Burlingame, CA). The reaction will be developed with the DAB peroxidase substrate reagent (Santa Cruz, CA). The reaction will be stopped after the negative control (a sentinel patient slide tissue will be treated with a 1:4000 dilution of pre-immune rabbit anti-GASP-1 serum) shows some color development. The slides will be rinsed in distilled H2O, counterstained with Gills Hematoxylin, rinsed and mounted with glycerol (Sigma, MI). Slides will be examined under bright field microscopy and microphotographs taken. Stain scores on a scale of 1-4 will be assigned to each case by a breast pathology expert at Temple University, in a blinded manner based on the tissue array position.

Figure 1 -Stained tumor section using the protocol described above**.** The stained section was photographed at 200x magnification.



Research Project 10: Project Title and Purpose

p38SJ, a Novel DING Protein from St. John's Wort Inhibits Tumor Cell Growth via Induction of Cell Cycle Arrest – p38SJ is a novel DING phosphatase isolated from St. John's Wort. Our preliminary data demonstrate that p38SJ inhibits proliferation of human T98G glioblastoma cells, as increased toxicity was observed upon treatment of cells with p38SJ. Furthermore, pre-treatment of rapidly proliferating U87MG cells with p38SJ leads to cell growth delay and induces cell cycle arrest in G0/G1 phase. Our observations provide evidence that p38SJ alters signaling pathways that impact cell growth and proliferation. The purpose of this project is to demonstrate that treatment of primary glioblastoma tumors from patients with p38SJ will result in the suppression of tumor growth. Thus p38SJ can be developed and used as an anti-cancer agent.

Anticipated Duration of Project

1/1/2011 – 12/31/2011

Project Overview

Recently we have purified a novel protein, p38SJ, from a callus culture of *Hypericum perforatum*. p38SJ has a modular structure highlighted by a phosphate-binding domain in the

central region of the protein. Our data indicate that p27SJ, a truncated form of p38SJ, exhibits phosphatase activity and, by that, controls cellular proteins which are important for cell cycle and cell proliferation including the key signaling molecule, Erk1/2. Accordingly, reduced level of ERK1/2 was observed in glioblastoma cells in the presence p27SJ. As a result, cell cycle progression was delayed in 27SJ-expressing cells. Our preliminary data demonstrate that treatment of human glioblastoma cells with p38SJ purified protein from plant, results in a rapid cell cycle arrest. Introduction of full-length p38SJ into T98G glioblastoma cells caused 80% decrease in cell growth in colony formation. Together, these results illustrate the efficacy of p38SJ in suppressing glioblastoma cell growth and provide evidence for the potential use of this protein in blocking proliferation of tumor cells. In light of these observations, we hypothesize that p38SJ, by virtue of its phosphatase activity, interferes with the operation of several cell signaling pathways involving ERK1/2, affecting cell cycle and cell proliferation. Thus, in this project we will:

Specific Aim 1: Study the effect of a novel human DING protein on proliferation of various primary tumor cells and identify the mechanism by which p38 affects tumor cell growth.

Specific Aim 2: Identify responsive region within p38SJ that exhibits growth inhibitory effect on tumors.

The outcome of this research will determine pathways of p38SJ-mediated tumor growth inhibition and provide a novel biological tool for developing new therapeutic approaches for the treatment and prevention of tumor-associated diseases.

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Expected Research Outcomes and Benefits

We expect that the treatment of primary brain glioblastoma tumors from patients with a novel plant protein, p38SJ will result in the suppression of the growth of aggressive brain tumors.

The outcome of this project will determine molecular pathways of p38SJ-mediated tumor growth inhibition and provide a novel biological tool for development of new therapeutic approaches for the treatment and prevention of tumor-associated diseases. If we demonstrate that the novel plant protein p38SJ induces growth arrest of primary glioma cell lines, *then in future work p38SJ will*

be tested for tumoricidal activity in human glioma models in preparation for human clinical trails to cure brain tumors.

Summary of Research Completed

We performed four series of studies to investigate suppressive effect of p38SJ on primary glioblastoma cell growth. Glioblastoma multiforme (GBM), the most aggressive malignant human primary brain tumor, has the worst prognosis of any CNS malignancy. Thus, novel therapeutic strategies, including protein therapeutics, have long been sought. Activation and upregulation of EGFR and ERK1/2 pathways promoting cell proliferation have been found in many glioblastoma tumors. Previously, we demonstrated strong tumor growth inhibitory effect by p38SJ/DING phosphatase, a novel member of DING family of proteins, derived from in vitro cultivated *Hypericum perforatum* calluses, on the malignant glioma cell lines, T98G and U-87MG by affecting the phosphorylation status of several key kinases controlling signaling pathways and cell proliferation. In this study, we have evaluated the effect of p38SJ/DING on three primary glioblastomas.

p38SJ expression causes a reduction in primary glioma cell survival. In one series of experiments we evaluated primary glioblastoma cells transfected with pEYFP-p38SJ or pCDNA6-p38SJ along with cells transfected with control empty vectors pEYFP-C1 or pCDNA6-A (Figure 1). Cell viability assay (Figure 1A), phase images of cells (Figure 1B) and. immunofluorescence microscopy of cells (Figure 1C) showed robust inhibition of cell growth in p38SJ-expressing cells (left panels but not in p38SJ-negative control samples (right panels)). Expression of YFP-p38SJ was observed in perinuclear region while YFP was found everywhere in cells. Our results showed that p38SJ/DING caused a strong reduction in glioma cell proliferation. Comparison of YFP and YFP-p38SJ transfected glioma cells over 3 days demonstrated a profound differential in growth between the two, as assessed by fluorescence under fluorescent microscopy (Figure 1C).

The observed growth inhibitory effect of p38SJ/DING is likely mediated by the downregulation of several serine/threonine protein kinases, including ERK1, AKT3, JNK1, HSP27 and RSK2, and upregulation of serine 9 phosphorylation of glycogen synthase kinase (GSK)-3α/β that resulted in the inactivation of these pro-survival proteins. GSK-3β is a critical regulator of nuclear factor NF-κB activity in cancers, and inhibition of GSK-3β is important in the treatment of tumors with constitutively active NFκB, whose expression and activation was also affected by p38SJ/DING. Thus, p38SJ/DING has the capacity to affect the phosphorylation status of serine/threonine protein kinases controlling signaling pathways and cell proliferation in primary glioblastomas (Figure 2A). Meanwhile, tyrosine protein kinases, such as FGF-R2, HGFR, IGF-1R, Jak3 and TXK, or EGF receptors, including Erb3, were also affected, but to a lower extent (Figure 2B).

Finally, the expression and phosphorylation of one of the downstream targets, pro-oncogenic myocyte enhancer factor 2 (MEF2) transcription factor, was significantly dysregulated in p38SJ-expressing glioblastoma cells. MEF2, as a transcription factor, controls genes responsible for cell division, differentiation, and death and is associated with neuronal, muscle development and

cancer by promoting the growth of cells. Interestingly, pro-survival pathways that were inactivated by p38SJ/DING in glioblastomas, are known to cause apoptosis in neurons.

These results suggest that p38SJ/DING, accompanied with its neuroprotective functions, can be developed into a novel therapeutic agent for the treatment of malignant gliomas.

Additional experiments are in progress to identify the responsive region within p38SJ that is important for anti-tumor activity of p38SJ, using p38SJ deletion mutants.

Materials and Methods

Cell culture. Human primary glioblastoma cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Life Technologies, Inc.) and antibiotics (100 units/ml penicillin and 10 µg/ml streptomycin) at 37°C in a humidified atmosphere containing 7% CO2. Cells were plated in 6 x well dishes at a density of 50,000 /well and cultured in 3 ml medium.

Transfections. Transient transfection assays were carried out with Lipofectamine 2000, according the manufacturer's protocol, using primary glioblastoma cells. In brief, 150,000 cells were plated on a 60 mm plate and grown overnight. Transfection was carried out with 0.5 µg of pMEF2-luciferase reporter construct, in the presence and absence of 1 µg of pEYFP-p38SJ, pEYFP-C1, pCDNA6-p38SJ or pCDNA6. At 16 h post-transfection, cell extracts were prepared and equal amounts of proteins were assayed for luciferase activity. Each transfection was repeated a minimum of three times with different plasmid preparations.

Plasmids. The pCDNA6-p38SJ and YFP-p38SJ plasmids (GenBank: AAW57408.2), pCMV-MEF-2-HA (a gift from Dr. M. Greenberg) were used for transfection. pEYFP-C1 vector was obtained from BD Biosciences Clontech and pCDNA6 Myc/His was purchased from Invitrogen, Carlsbad, CA, USA.

Cell Viability Assay. 50,000 of primary glioblastoma cells, transfected with either pEYFP-C1 or YFP-p38SJ expressing plasmids were plated into 60 mm dishes. Each plate was inspected under fluorescent microscopy for expression of YFP and YFP-p38SJ, and the total number of cells counted.

Phospho-MAPK array. A human phospho-MAPK array kit was used from R&D Systems, Inc. (Minneapolis, MN). pEYFP-C1 and YFP-p38SJ transfected glioblastoma cell lysates were applied and processed over the phospho-MAPK array that allows the analysis of 38 MAPK kinases in cell extracts per the manufacturer's protocol.

Human Receptor Tyrosine Kinases (RTK) Antibody Array, Human Adipokine Antibody Array, and EGF Receptors Phosphorylation Array were used to examine the effect of p38SJ on the Serine or Tyrosine phosphorylation of signaling molecules (RayBiotech).

Western-blot (immunoblot) analysis. For immunoblot assay 25 micrograms of proteins in Laemmli sample buffer were heated at 95°C for 10 min and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to supported nitrocellulose membranes for 16 h at 40 mAmps at 40C. Proteins were visualized with the enhanced chemiluminescence detection system, ECL+ according to manufacturer's instructions (GE Healthcare, Piscataway NJ), and exposed to X-ray film.

Antibodies. Antibody specific for p38SJ (anti-p27SJ rabbit polyclonal antibody) and cell cycle proteins were obtained from Lampire Biological Laboratories, Inc. Pipersville, PA). Anti-HA antibody was purchased from Invitrogen (Invitrogen, Carlsbad, CA). Anti-phospho-MEF2 rabbit

polyclonal antibody and anti-MEF2 monoclonal mouse antibody and GRB2 were purchased from Santa Cruz Biotechnology (Santa Cruz Biotechnology, Inc., Santa Cruz, CA)
Microscopy. Fluorescence images were captured using an inverted fluorescent Nikon microscope with deconvolution software (SlideBook 4.0.1.34; Intelligent Imaging, Denver, CO, USA), and confocal microscope.

Publications:
Markus J. Bookland, M.D.; Nune Darbinian, Ph.D.; Michael Weaver, M.D.; Shohreh Amini, Ph.D.; Kamel Khalili, Ph.D. *Growth Inhibition of Malignant Glioblastoma by DING Protein*. J of Neurooncology. J. of Meurooncology, 2011 (accepted).

Tarek Khader, Markus Bookland, MD, Michael Weaver, MD, Nicholas Hoffman, Madesh Muniswamy, PhD, Michael Greenberg PhD, Shohreh Amini, PhD, Kamel Khalili, PhD, Nune Darbinian, PhD. *Growth Inhibition of Primary Glioblastoma by p38SJ/DING phosphatase: Dysregulation of MEF2*. Dawn Marks research day poster presentation, June 4 2011

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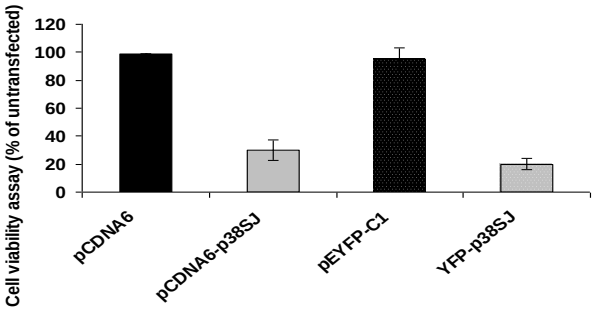

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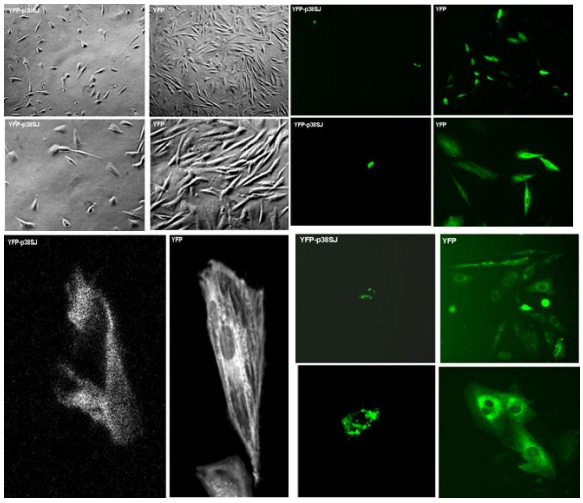

Figure 1. *p38SJ inhibits cell proliferation*.
(A) Cell viability assay using primary glioblastoma cells transfected with p38SJ. The quantitative representation of cell viability assay is displayed after 3 days. Error bars represent the standard deviation from three independent experiments. Controls were set as 100 (%).
(B) Phase and fluorescence
(C) Images of primary glioblastoma cells transfected with pEYFP-C1 and pYFP-p38SJ.

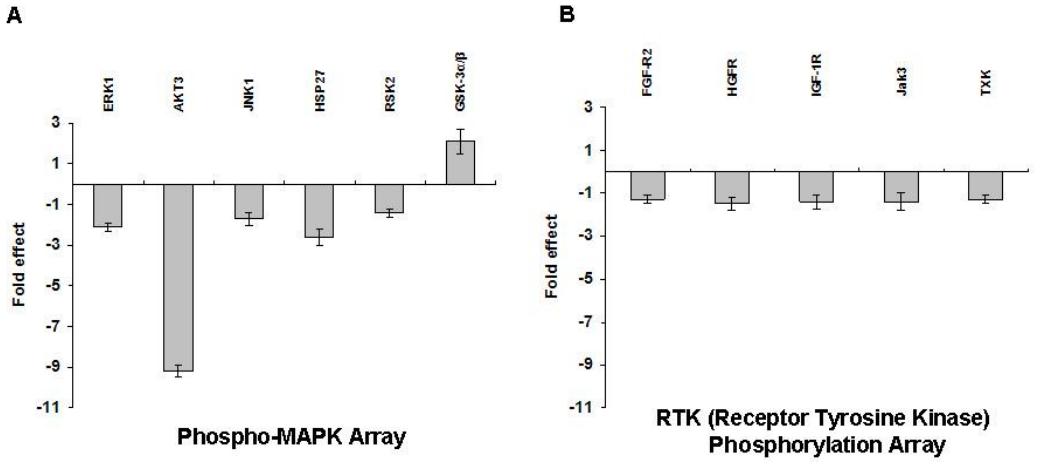

Figure 2. *p38SJ inhibits Serine/Threonine phosphorylation. Effect of p38SJ on Serine/Threonine or Tyrosine phosphorylation of MAPK signaling and Human Receptor Tyrosine Kinases (RTK) in primary glioblastoma cells.*
(A) Phospho-MAPK array using cell lysates from cells transfected with YFP and YFP-p38SJ. Quantitative representation of phospho-MAPK protein levels in p38SJ-expressing cells as determined by densitometric analysis of each spot. The values were normalized to the levels seen in YFP expressing cells. As indicated, p38SJ inhibits Serine/Threonine phosphorylation of key MAPK signaling molecules. (B) RTK antibody array. p38SJ slightly inhibits Tyrosine phosphorylation of Receptor Tyrosine Kinases.

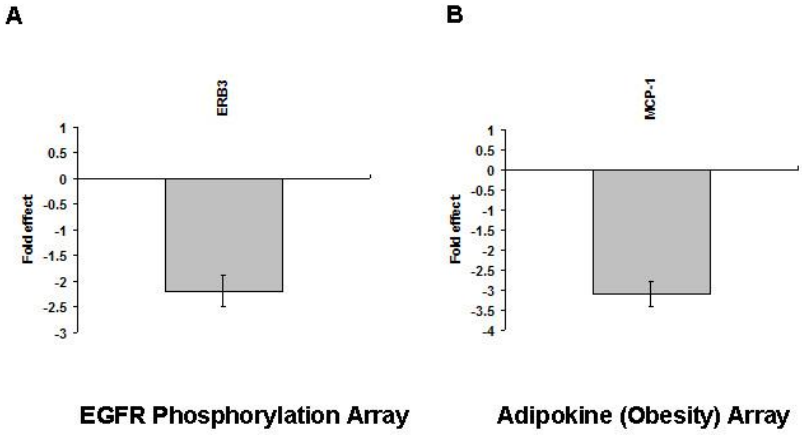

Figure 3. *p38SJ inhibits EGFR family of membrane receptors.*
(A) Erb3 expression and and MCP-1 secretion (B) are affected in p38SJ-expressing primary glioblastoma cells.

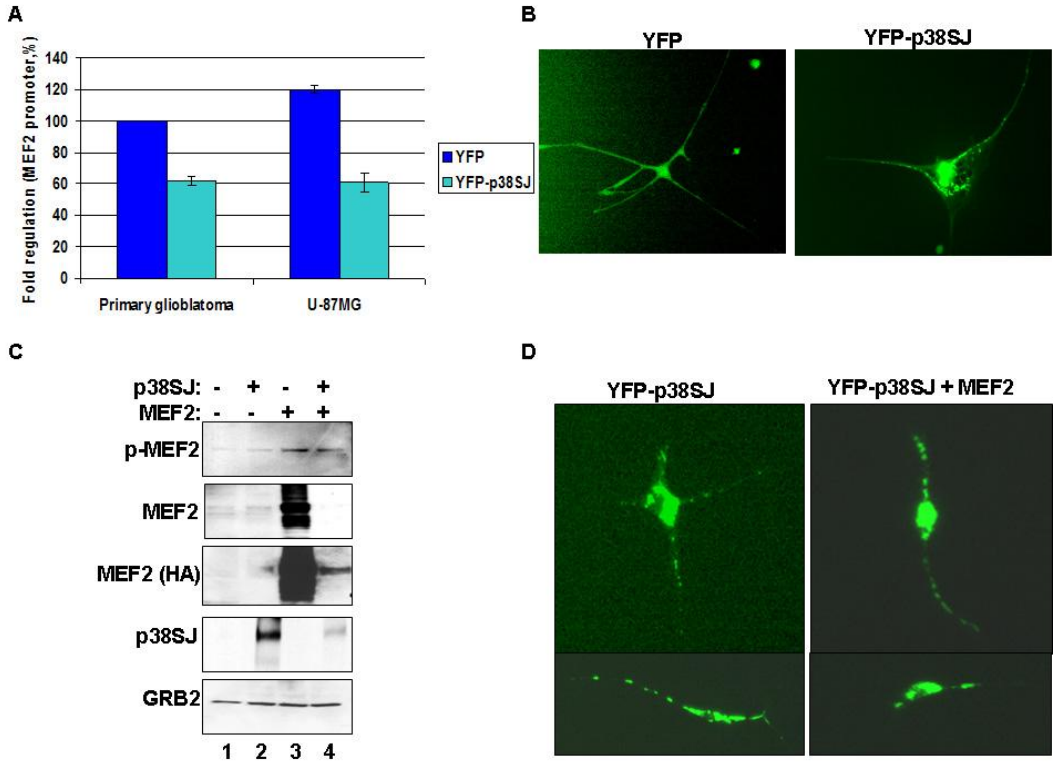

Figure. 4. *p38SJ dysregulates MEF2 in glioblastoma cells.*
(A) The effect of p38SJ on the promoter activity of the MEF2 was examined using the MEF2-luciferase reporter construct in the glioblastoma cells. (B) Expression of YFP-p38SJ and YFP cells that were stadied in promoter-luciferase assay. (C) Effect of p38SJ on MEF2 protein expression and stability. Western blots analysis of extracts from YFP-p38SJ-expressing cells with or without MEF2 for phospho-MEF2 (top panel), total MEF2 (second panel), or exogenously expressed MEF2 (third panel). Grb2 served as a loading. (D) MEF2 inhibits formation of processes, number and length of processes in glioblastoma cells.