Cannabigerol (CBG) is a non-psychoactive phytoannabinoid isolated from cannabis. The aim of this study was to measure the modulation of CBG on the effects of several synthetic and endocannabinoid agonists on the human CB2 cannabinoid receptor stably expressed in HEK293 cells. A homogenous time-resolved fluorescence method was used to quantify cannabinoid-induced, CB2-mediated inhibition of cyclic adenosine monophosphate (cAMP) levels. At concentrations up to 10 μM, CBG by itself had no effect on forskolin-stimulated cAMP accumulation. Furthermore, CBG did not significantly modify cAMP inhibition induced by synthetic cannabinoids CP-55,940, HU-210, or endocannabinoid 2-arachidonylglycerol (2-AG). However, CBG was found to increase the efficacy of endocannabinoid anandamide (AEA). Taken together, these results demonstrate that CBG is neither an orthosteric agonist nor an antagonist at the CB2 receptor. In addition, these data suggest that CBG possibly changes the efficacy of AEA on CB2 receptor via metabolic modulation.

**Abstract**

Cannabigerol (CBG) is a non-psychoactive phytoannabinoid isolated from cannabis. The aim of this study was to measure the modulation of CBG on the effects of several synthetic and endocannabinoid agonists on the human CB2 cannabinoid receptor stably expressed in HEK293 cells. A homogenous time-resolved fluorescence method was used to quantify cannabinoid-induced, CB2-mediated inhibition of cyclic adenosine monophosphate (cAMP) levels. At concentrations up to 10 μM, CBG by itself had no effect on forskolin-stimulated cAMP accumulation. Furthermore, CBG did not significantly modify cAMP inhibition induced by synthetic cannabinoids CP-55,940, HU-210, or endocannabinoid 2-arachidonylglycerol (2-AG). However, CBG was found to increase the efficacy of endocannabinoid anandamide (AEA). Taken together, these results demonstrate that CBG is neither an orthosteric agonist nor an antagonist at the CB2 receptor. In addition, these data suggest that CBG possibly changes the efficacy of AEA on CB2 receptor via metabolic modulation.

**Background**

Very little has been published regarding CBG’s binding capabilities to the cannabinoid receptors. Even less has been published regarding agonism or antagonism of the receptors by CBG. It has been shown that CBG binds with low affinity to both the CB1 and CB2 receptors, with slightly higher affinity for CB1 [1,2,3]. One group has shown that CBG antagonizes the CB1 receptor, but states that further study is needed for CB2 receptor agonism/antagonism [1].

**Specific Aims**

1. Determine if CBG is an agonist/antagonist for the CB2 receptor, and determine if CBG modulates the effect of other cannabinoid agonists on CB2 using an HTRF cAMP assay.
2. Determine if CBG binds orthostERICally to the CB2 receptor using a competition binding assay.
3. Determine if CBG binds allosterically to the CB2 receptor using a dissociation kinetic assay.
4. Determine if CBG modulates anandamide degradation using thin layer chromatography (TLC).

**References**

BACKGROUND:

Globally, esophageal cancer is the sixth leading cause of cancer death and is the eighth most frequent tumor [1]. Esophageal cancer is classified by its high mortality rate and unfavorable prognosis. Tobacco use, alcohol consumption, gene mutations, age, gender and obesity are associated risk factors [3]. There are two major types of esophageal cancer: adenocarcinoma and esophageal squamous cell carcinoma (ESCC). Although there are more cases of ESCC worldwide, esophageal adenocarcinomas are increasing in the United States and other developing countries [1].

The cause(s) of esophageal cancer are unknown. Recently, however, ESCC has been linked to an oral bacterium, Porphyromonas gingivalis. P. gingivalis is a Gram negative, asaccharolytic anaerobe which is recognized as a keystone pathogen in periodontitis. P. gingivalis has also been associated with other cancers including gastric cancer, pancreatic cancer and oral squamous cell carcinoma. From a mechanistic perspective, P. gingivalis can cause changes to both cell division and apoptosis in eukaryotic cells. P. gingivalis demonstrates anti-apoptotic activity in primary gingival epithelial cells by controlling the Jak/Stat3 signaling pathway. This can lead to the up-regulation of miR-203 which can suppress apoptosis [4]. P. gingivalis can also change the progression of the cell cycle by altering CDK (cyclin-dependent kinase) activity and decreasing the level of the p53 tumor suppressor [2]. In addition, P. gingivalis secretes a nucleoside diphosphate kinase (NDK), which can act as an ATPase and suppress ATP dependent kinase (CDK) regulation of miR-7 receptors [2].

The purpose of this study was to examine the effects of different strains of P. gingivalis on chemically induced apoptosis in esophageal epithelium tumor cells. We hypothesize that infection of P. gingivalis will impinge on the Camptothecin (CAMP)-induced apoptosis of KYSE-30 cells, a typical esophageal squamous cancer cell line.

MATERIALS & METHODS:

- P. gingivalis strains, ATCC 33277 and NDK-deficient P. gingivalis 33277, were cultured in GAM broth (Gifu Anaerobic Medium). Cells were grown anaerobically at 37°C.
- Esophageal cancer cell line, KYSE-30, was cultured using RPMI-1640 with 10% FBS. P. gingivalis in late log phase growth was infected into the cancer cells at a MOI of 1:1.
- 20h after the epithelial cells were infected with P.gingivalis, Camptothecin was used to induce apoptosis in the cells. After 4h of Camptothecin incubation, apoptotic cell death in esophageal cancer cells was assayed by PE Annexin V/Dead Cell Apoptosis Kit with SYTOX Green® for flow cytometry.

RESULTS:

<table>
<thead>
<tr>
<th>Unstimulated</th>
<th>Camptothecin (CAMP)</th>
<th>P. gingivalis 33277 (Pg)</th>
<th>P. gingivalis NDK/- P. gingivalis NDK +CAMP</th>
</tr>
</thead>
</table>
|              |                     |                         | Suppression of apoptosis in esophageal cancer cells by Porphyromonas gingivalis

Maya C. McFrazier, Xiaoxian Duan, Diane E. Renaud, David A. Scott & Huizhi Wang
Department of Oral Immunology and Infectious Diseases, University of Louisville School of Dentistry

CONCLUSIONS:

- NDK gene deficiency abrogates the ability of P. gingivalis to suppress Camptothecin-induced apoptosis in KYSE-30 cancer cells, suggesting NDK is essential for the anti-apoptotic effects of P. gingivalis.
- Infection of P. gingivalis in esophageal squamous cancer cells may represent a biomarker for this disease.
- P. gingivalis infection in ESCC patients could be a prognostic factor for overall survival (supported by other unpublished data).
- Eradication of P. gingivalis could potentially contribute to a reduction in the overall ESCC burden.

FUTURE DIRECTIONS:

Future studies will aim to elucidate the molecular mechanism responsible for the anti-apoptotic ability of P. gingivalis and to develop molecular intervention strategies for alleviating the progression of esophageal cancer.

REFERENCES:


ACKNOWLEDGEMENTS:

This study was supported by grants (R25-CA134283, NCI R25 grant, University of Louisville Cancer Education Program NIH/NCI; and DE 023633 (HW), DE 017680 (DAS) from National Institute of Dental and Craniofacial Research, NIH.
A Multi-Organ Study Using Microwave: A Comparison of the Solero system to the Sulis V pMTA and the NeuWave Certus 140 systems

Robert CG Martin II, MD, PhD, Rachel O’Connor

Department of Surgery, Division of Surgical Oncology, University of Louisville, Louisville, KY

Introduction

• Microwave ablation is designed to deliver a controlled transmission of electromagnetic energy into a targeted tissue during a medical procedure.
• Typically, the procedure is performed percutaneously or laparoscopically which provides rapid recovery, shorter hospital stays and immediate improvements without an open incision.
• This study was performed under GLP guidelines to evaluate and establish the equivalence of the AngioDynamics Solero Microwave Ablation System.
• 15 swine underwent 45 ablations in a combination of the liver, lung, and kidney organs.

Purpose of Pilot Study

• The goal of this GLP study is to evaluate and establish substantial equivalence of the Solero system to the Sulis V and NeuWave systems with respect to safety and effectiveness.
• The track ablation feature will be tested to establish safety and efficacy.

Experimental Design

• Protocol was approved and IACUC application was accepted.
• Copies of SOPs for animal husbandry, veterinary care, CII laboratory procedures and associated equipment were all organized and filed before the start date of the study.
• The pig tissue and organ size and consistency closely model those of humans.
• 3 groups were assigned: laparoscopic, percutaneous, and a “back-up”.
• Each pig had predetermined ablation locations, wattages, and time.
• The pigs underwent appropriate acclimation time upon arriving at CII. The pigs undergo ablation surgery and receive MRI (day 1).
• After 28 days, the pigs receive second MRI to see efficacy of ablations.
• They then undergo necropsy and histology of ablated organs.

Results

• Thus far, a total of 8 female pigs have been ablated with 7 in current stable condition.
• One pig has undergone emergency necropsy.
• All of the machines have been easy to use with user-friendly interfaces for easy set-up.
• The efficacy of the Solero system appears to match that of the Sulis V and NeuWave systems.

Conclusions

• This study has given promising data that the Solero system will perform as well as the Sulis V and Certus 140.
• Track ablation feature in the Solero system is effective.

Grants

• National Cancer Institute grant R25- CA134283
Psychological Distress and Malnutrition Biomarkers are associated with Head and Neck Cancer Progression and Survival

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1Dept of Otolaryngology-Head and Neck Surgery & Communicative Disorders; 2University of Louisville School of Medicine; 3Dept of Psychological & Brain Sciences; 4James Graham Brown Cancer Center; University of Louisville, Louisville, KY

Abstract

We previously reported that depressive symptoms predict greater likelihood of interruption and incomplete response to treatment in head and neck cancer (HNC). Here we extend those examinations to two-year disease-free and overall survival. Further, the relationship between depressed mood and poor appetite, HNC patients are at high risk for cachexia. We hypothesized that greater psychological symptoms and malnutrition biomarkers would be associated with increased weight loss, and poorer two-year disease-free (DFS) and overall survival (OS).

Methods & Results

A Model of Cancer Cachexia

<table>
<thead>
<tr>
<th>Description</th>
<th>Score Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distress Thermometera</td>
<td>0-5</td>
</tr>
<tr>
<td>Hospital Anxiety and Depression Scoreb</td>
<td>0-4</td>
</tr>
<tr>
<td>Albumin</td>
<td>&lt;3.5 g/dL</td>
</tr>
<tr>
<td>Hypoalbuminemia</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>&lt;12.0 g/dL</td>
</tr>
<tr>
<td>AST</td>
<td>1-34 U/L</td>
</tr>
<tr>
<td>ALT</td>
<td>&lt;40 U/L</td>
</tr>
</tbody>
</table>

Tumor Growth

Patients presenting to a Multidisciplinary Head and Neck Clinic with a primary HNC diagnosis from July 2012 and August 2013 completed DT and HADS. Biomarker levels were gathered from routine laboratory workup. Weight change was calculated in kg using pre- and post-treatment values. Two-year DFS and OS data were gathered from medical records. Distress scores and biomarkers of cachexia were entered as predictors, with weight loss, and two-year DFS and OS entered separately as outcome variables, in hierarchical and Cox regressions adjusted for patient age at diagnosis, cancer stage, site of disease, and treatment regimen.

Conclusions

• This study identifies a cohort of HNC patients at risk for poorer progression and survival outcomes.
• Depressive symptoms are associated with a greater likelihood of poorer short-term (treatment interruption and incomplete response) and long-term (overall survival) outcomes in this sample of HNC patients.
• Lower pretreatment hemoglobin was associated with poorer overall survival for males and females (HR=0.740, 95%CI=.561–.977, p=.033).
• Malnutrition biomarkers should be further examined to determine their validity as predictors of cachexia and long-term outcomes.
• Future studies should examine biological (e.g., inflammatory, immunologic) factors with the potential to mediate the relationships between psychosocial symptoms and cancer outcomes.

Introduction

Cancers of the head and neck (HNC) account for approximately 5% of all malignancies.1 HNCs are associated with significant distress, anxiety, and, depression.2,3 The extent of emotional distress may be associated with disease characteristics,4 as tumors located on a patient’s face or mouth may interfere with daily tasks such as speaking or swallowing, as well as changes to body image and self-esteem, all contributing to negative emotional outcomes.

Cachexia is defined as a 5% loss of total body weight.4 HNC patients are at high risk for cachexia due to location of disease and related symptoms. Cancer cachexia may be characterized by loss of both adipose and muscle tissue, anorexia, and anaemia. At diagnosis, 35%-69% of HNC patients are malnourished due to tumor-related malnutrition.5-8 Cachexia may also have systemic effects that influence tumor growth and response to treatment, including alteration of immune function.9 Commonly measured serum biomarkers, including albumin, AST, ALT, and hemoglobin, serve as indicators of a patient’s systemic nutritional status.10

The impact of cachexia may be significant, potentially increasing risk for poorer disease-free and overall survival.8 Indeed, cachexia has been related to 20% of HNC-related deaths.8,11 Early detection of cachexia risk factors may therefore help prevent poor outcomes.12 Psychosocial stressors and biomarkers of cachexia should be evaluated at or near the time of diagnosis. Early detection may allow more opportunity for psychological and medical intervention.

We hypothesized that greater psychological symptoms and malnutrition biomarkers would be associated with increased weight loss, and poorer two-year disease-free (DFS) and overall survival (OS).

References

Impact of Quercetin on miR-21 Cellular Proliferation and Migration in Metastatic and Non-Metastatic Prostate Cancer

Thomas Packer B.A.1, Dominique Jones M.S.1 and LaCres P. Kidd2,3
Department of Pharmacology and Toxicology1 and James Graham Brown Cancer Center2

RESULTS

QUANTIFICATION OF MALIGNANT PROSTATE CELL LINES

Patients diagnosed with metastatic disease are typically non-responsive to conventional treatment strategies. Consequently, new biomarkers and chemoprevention strategies are needed for the effective treatment of aggressive and potentially lethal forms of PCA.

Role of miRNAs as Prostate Cancer Biomarkers

miRNAs (miRNA), short non-coding, stranded RNAs, may serve as effective tools to improve cancer diagnosis, prognostic, clinical management, and prevention strategies. Current studies suggest miRNAs are upregulated in prostate cancer in silico. miRNAs function as oncogenes or tumor suppressors that regulate the expression of genes linked to cell proliferation, apoptosis, differentiation, metastasis and angiogenesis.

Recent evidence in the literature suggest miR-21 was overexpressed in tumors from collected European-American men diagnosed with prostate cancer relative to disease-free individuals. The over-expression of oncomiRNA, such as miR-21 may be counteracted by various chemopreventive agents such as Quercetin.

Quercetin as a chemopreventive agent

Quercetin is a flavonoid found in fruits (cranberry, black plums, strawberries, grapes, apples), vegetables (kale), leaves (e.g., radish, fennel), herbs (dill, cilantro), grains (e.g., buckwheat) and red wine.

Quercetin is an anti-oxidative, anti-inflammatory, anti-cancer properties. Recent studies reveal quercetin inhibits cell invasion, migration, and proliferation in immortalized and metastatic cell lines as well as modulates expression of DNA repair, extracellular matrix degradation and tumor invasion, angiogenesis, apoptosis and cell cycle genes.

A few in vitro studies indicate quercetin may alter the expression of miRNAs. However, it is not clear whether quercetin may reduce the expression of miR-21 and aggressive cancer behavior using prostate cancer cell lines (PC-3 and E006AA).

OBJECTIVES

To evaluate whether quercetin may modulate the expression of miR-21 using two prostate cancer cell lines, namely E006AA (primary cell line derived from an Aa) and Castrates (metastatic line).

Assess the impact of quercetin treatment on cell proliferation and migration in lines transiently transfected with miR-21.

HYPOTHESIS

Quercetin will decrease cell proliferation and migration in the non- and metastatic prostate cancer cell lines. Quercetin treatment will decrease the expression of oncomiRNA miR-21.

CLINICAL RELEVANCE

The findings of our study may serve as a foundation for future studies that seek to identify and validate new chemopreventive strategies effective modulation of oncomiRNA and treatment of pre- and metastatic prostate cancer.

DISCUSSION & CONCLUSIONS

Baseline Levels of miR-21 in Prostate Cancer Cell Lines

miR-21-3p was significantly up-regulated in PCs compared to normal control cell lines (RWPE1).

However, miR-21-5p expression was not differentially expressed in E006AA and PCs compared to RWPE1 cells.

Impact of Quercetin on Cells Proliferation In Vitro

Relative to the vehicle control (0.0375% DMSO)
- Cell population of PC3 cells was decreased significantly by quercetin treatment (25-75µM) within 24h and 48h time points

Cell proliferation of E006AA cells was decreased significantly by quercetin (50-75µM) within 24h and 48h time points.

Quercetin Effective Concentration In Vitro

The EC50 for E006AA and PC3 was calculated as 31.9-39.9µM and 39.4-23µM for 24-48hrs, respectively.

Impact of Quercetin on Cell migration using a Wound Healing Assay

Significant 18.5% decrease in cell migration was observed with Quercetin EC50 treatment compared to vehicle control in both PC3 and E006AA cells after 12hr and 24hr time points.

We also demonstrated a 23% and 14% decrease in cell migration in PC3 and E006AA cells with ectopic expression miR-21-3p following treatment with the Quercetin EC50.

Impact of Quercetin on Cell Proliferation using the BrdU Assay

Modest decrease in cell proliferation was observed in PCs treated with EC50 and miR-21-3p mimic.

No significant differences were between any of the treatment groups for E006AA cells

FUTURE DIRECTIONS

- Modify cell proliferation and cell migration assays using a wider quercetin dosage range (2.5µM-150µM) and lower %DMSO (i.e., 0.01%)
- Determine whether quercetin treatment with other quercetin metabolite or quercetin analogs can down-regulate the expression of tumor suppressing miRNAs using next generation sequencing.
- Reduce aggressive PCA phenotypes (i.e., cell proliferation, colony formation, cell invasion) using metastatic PCA (i.e., LNCAP, DU145, MDA-PCA-2A, MDA-PCA-30).
- Reduce tumor size, tumor number, or metastasis using animal models.
- Assess whether quercetin can modulate the expression of human miRNAs and/or PCA phenotypes.
- Evaluate whether quercetin treatment combined with conventional drug treatments may help increase survival rates among pre- or metastatic PCA patients.

ACKNOWLEDGEMENTS

The wound healing assay was developed by Barbara Safiejko-Mrozeka (University of Oklahoma) and maintained in Dr. Brian Ceresa’s lab.

Il NCI R25 Cancer Education Grant to D.W. Hein (CA134283).

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Temozolomide Enhances Breast Cancer Virotherapy Regardless of Estrogen Receptor Status

Rigoberto Perez-Hernandez 1, Heshan Sam Zhou1,2, Rajesh Sharma2, Kelly M. McMasters1,2, and Jorge G. Gomez-Gutierrez1,2

1The Hiram C. Polk MD Department of Surgery and 2James Graham Brown Cancer Center, University of Louisville, School of Medicine, Louisville, KY, 40202.

Introduction

Oncolytic virotherapy has made significant progress in recent years; however, widespread approval of virotherapeutics is still limited. Primarily, this is due to the fact that currently available virotherapeutics are mostly tested in monotherapeutic clinical trials exclusively (i.e., not in combination with other therapies) and so far have achieved only small and often clinically insignificant responses. For this reason, combination strategies of virotherapy with highly genotoxic regimens, such as chemotherapy, are of major interest. Therefore, in this study we investigate whether Tamoxifen (TAM) or Temozolomide (TMZ) in combination with an oncolytic adenovirus (Adhz60) could enhance virotherapy effectiveness in human and murine breast cancer (BC) cells.

It was found that TAM increased Adhz60 mediated-cytotoxic effect (CPE) only in MCF-7 cells; in contrast, TMZ enhanced Adhz60 mediated-oncolysis in all breast cancer cells evaluated here. It seems that TAM increased BC virotherapy is limited to estrogen receptor (ER)-positive cells, whereas TMZ enhanced BC virotherapy effectiveness is independent of ER status.

The clinical relevance of this finding is that the combined therapy of oncolytic adenovirus with TMZ could be applied in clinical settings for patients with either types of BC cells: ER-positive or -negative.

Conclusions

This study provides evidence that TAM efficiently enhances oncolytic virotherapy effectiveness in MCF-7 ER-positive cells. However, this increased virotherapy is likely restricted to ER-positive cells.

Interestingly, TMZ a drug commonly used to treat melanoma and glioblastoma was able to enhance virotherapy potency in human and murine breast cancer cells. Most importantly, TMZ increases oncolytic virotherapy effectiveness independent of ER status in breast cancer cells.

In this study, it was also found that the combination therapy of oncolytic adenovirus (Adhz60) with TMZ resulted in a synergistic cancer cell killing effect.

The clinical relevance of the combined therapy of oncolytic adenovirus with TMZ is accentuated by the fact that breast tumors from patients with either ER-positive or -negative cells could be equally destroyed, which represents a more wide therapy for breast cancer.

Results

Fig. 1 A) Crystal violet staining to evaluate the Adhz60 cytopathic effect in BC cells. B) MTT assay to assess the cytotoxic effect of TAM or TMZ in BC cells. (72h post treatment)

Conclusions

This study provides evidence that TAM efficiently enhances oncolytic virotherapy effectiveness in MCF-7 ER-positive cells. However, this increased virotherapy is likely restricted to ER-positive cells.

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The clinical relevance of the combined therapy of oncolytic adenovirus with TMZ is accentuated by the fact that breast tumors from patients with either ER-positive or -negative cells could be equally destroyed, which represents a more wide therapy for breast cancer.

Fig. 2 Evaluation of the Adhz60 mediated-cytotoxic effect alone or in combination with TAM or TMZ in BC cells. (72h post treatment)

(A) MCF-7 ER-positive
(B) MDA-MB-231 ER-negative
(C) 4T1 murine ER-negative

Acknowledgements

Research supported by the National Cancer Institute grant R25-CA134283 and the University of Louisville School of Medicine Cancer Education Program (R. P. H.).
Establishing a Link Between Ubiquilin and SUMO
Cody R. Sheffield and Levi J. Beverly
Department of Pharmacology and Toxicology
University of Louisville School of Medicine

Introduction
Post-translational modifications often dictate the fate of a newly synthesized protein. These modifications, or lack thereof, can result in an activation or deactivation of a protein, and many other functions. One of these modifications is a family of proteins called small Ubiquitin-like modifiers (SUMO). These proteins have a vast array of functions including: nuclear transport, assisting with apoptosis, and protein stability, among others.

This project was an attempt to look at the role that SUMO proteins play, if any, in the protein Ubiquilin. This protein was chosen for a variety of reasons. For example: Our has shown that loss of Ubiquilin results in cell proliferation, as well as epithelial-mesenchymal transition (EMT), which is a process observed in cancer. Our lab has previously shown that Ubiquilin function has been lost in a large percentage of certain cancer cell types. These results suggest an important role for Ubiquilin in cancer biology. Interestingly, SUMO proteins also play a role in the inhibition of EMT, possibly suggesting that loss of Ubiquilin function is due to SUMOylation, or a problem with the SUMOylation pathway for Ubiquilin.

Background information
Ubiquilin function is lost in many cancer cell lines. Ubiquilin has a Ubiquitin-like domain and a Ubiquitin-associated domain. As seen in the above diagram, SUMO is an antagonist of Ubiquitin, meaning that they bind to the same lysine residues and that they may interact.

Methods
To establish this link between SUMO and Ubiquilin, we co-transfected 293T cells with HA epitope tagged SUMO, and FLAG epitope tagged PCS2 PLC1 (Ubiquilin). We then immunoprecipitated the lysates created from these cells with FLAG beads, and subsequently used Western Blot analysis. The FLAG beads attach to anything with the FLAG epitope (Ubiquilin) and separate it and anything attached to it from other cellular materials. Then, by using the HA antibody, we used western blotting to detect whether the HA epitope tagged SUMO proteins were separated with Ubiquilin or not.

Hypothesis
Our hypothesis is that Ubiquilin, or something that interacts with Ubiquilin, is SUMOylated. This SUMOylation could either result in the activation or deactivation of Ubiquilin. It could also result in the activation or deactivation of something bound to Ubiquilin that could change the function of Ubiquilin itself.

Results

Conclusion/Future work
Unfortunately this data provides no conclusive evidence either for or against the hypothesis. This experiment should be repeated in the future. Also, we plan to block the proteasome in 293T cells expressing transfected SUMO and use western blotting to determine any difference in SUMO expression between cells with proteasomal blockage and normal 293T cells.

Acknowledgements
Research supported by a grant from the National Cancer Institute’s grant R25 CA 134283 and the University of Louisville Dept. Pharmacology and Toxicology.

I would also like to thank Dr. Beverly and the members of our lab.