Cannabigerol Modulates the Efficacy of Anandamide On the CB2 Cannabinoid Receptor

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Abstract

Cannabigerol (CBG) is a non-psychoactive phytocannabinoid 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 homogeneous 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-arachidonoylglycerol (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).

Results

Figure 1: Effect of CBG on Forskolin- stimulated cAMP accumulation

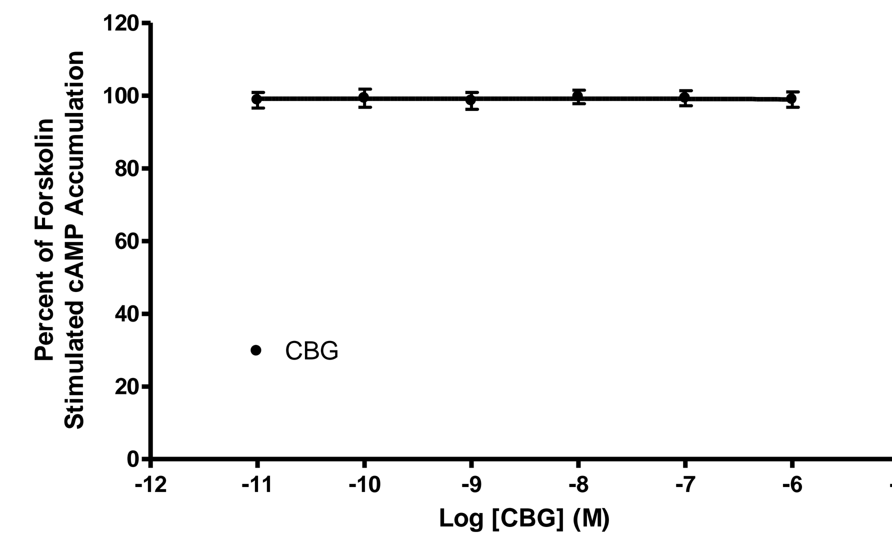


Figure 2: Effect of CBG on forskolin- stimulated cAMP accumulation by known cannabinoid agonists

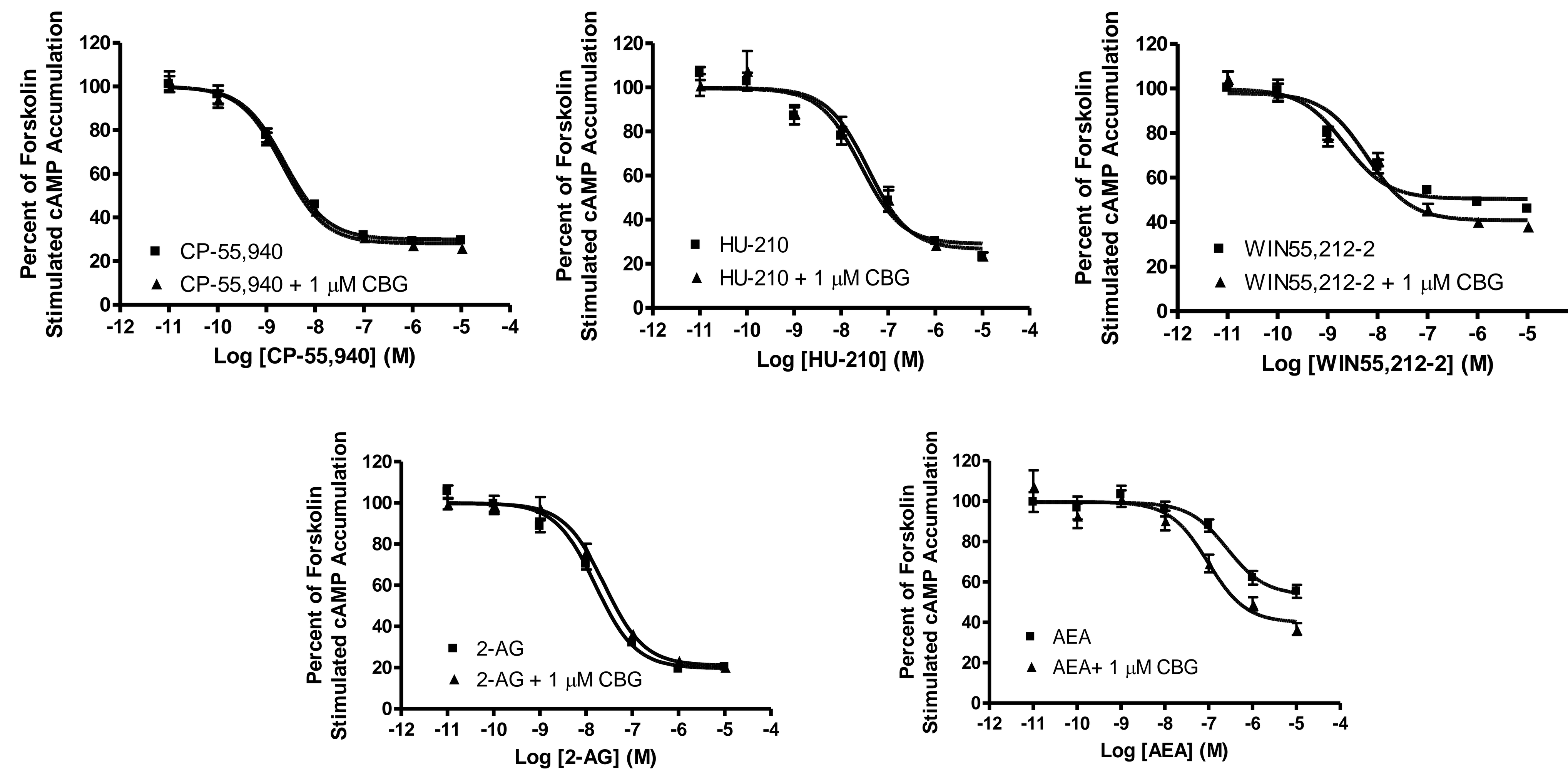


Figure 3: Competition of [3H]WIN55,212-2 binding by CBG

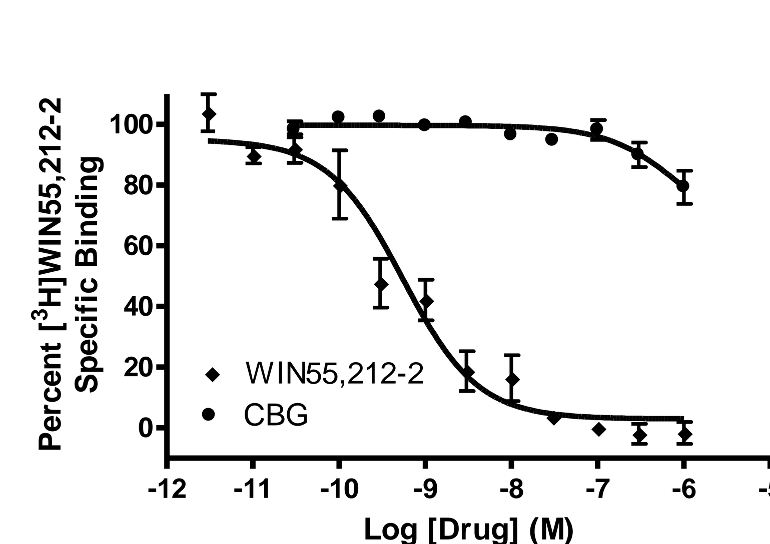


Figure 4: Effect of CBG on [3H]WIN55,212-2 dissociation from the CB2 receptor

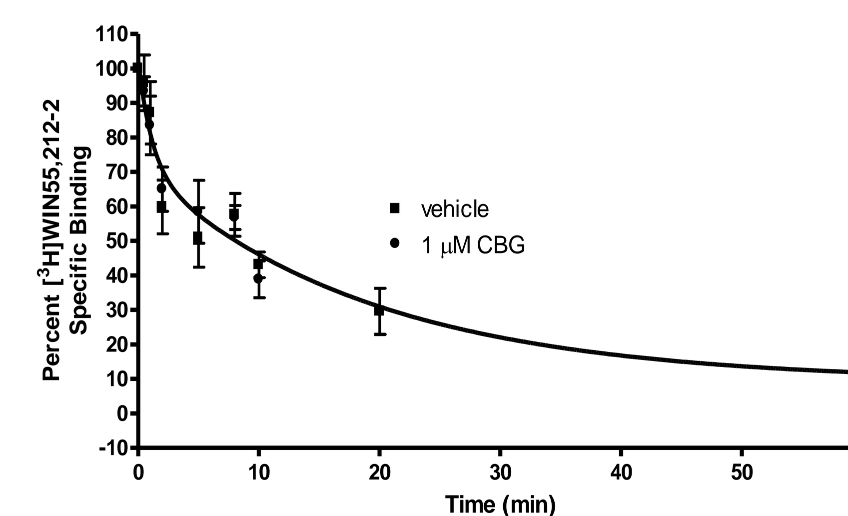
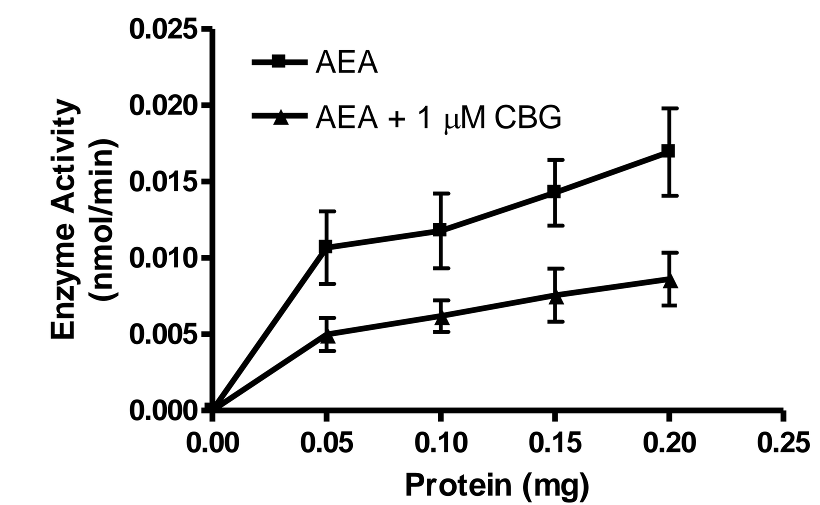


Figure 5: Effect of CBG on Anandamide degradation



Conclusions

1. CBG is not an agonist or antagonist for the CB2 receptor, but potentiates the effect of anandamide on CB2.
2. CBG binds to the orthosteric site of CB2 with low affinity, and does not bind allosterically.
3. CBG reduces AEA degradation, which may explain increase in efficacy observed in the cAMP assay.

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Suppression of apoptosis in esophageal cancer cells by *Porphyromonas gingivalis*

UNIVERSITY OF LOUISVILLE

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BACKGROUND:

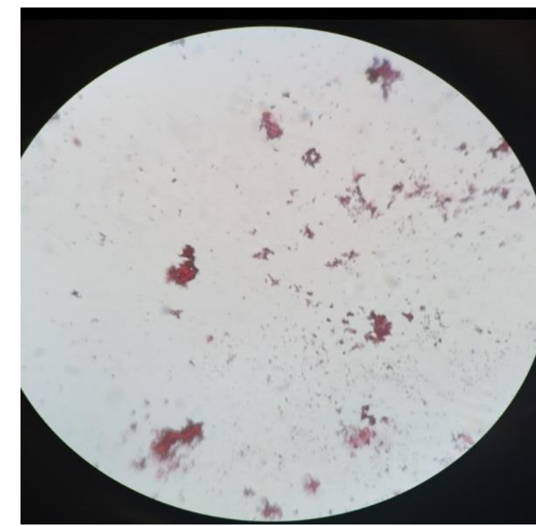
Globally, esophageal cancer is the sixth leading cause of cancer death and is the eighth most frequent tumor [1]. Esophageal cancer is classified by its high mortality rate and unfavorable prognosis. Tobacco use, alcohol consumption, gene mutations, age, gender and obesity are associated risk factors [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/Akt/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 apoptosis through the $P2X_7$ receptors [2].

The purpose of this study was to examine the effects of different strains of *P. gingivalis* on chemically induced apoptosis in esophageal epithelia 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 10:1.
- 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.



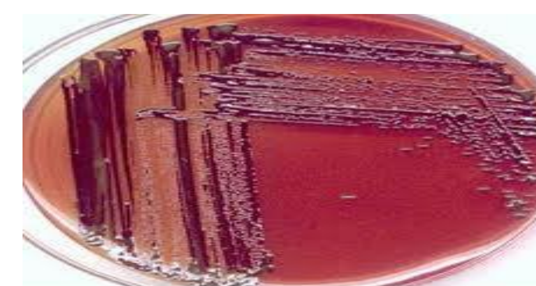


Figure 1: Gram stain of *P.gingivalis* ATCC 33277 (top). *P.gingivalis* cultured on a GAM blood agar plate (bottom).

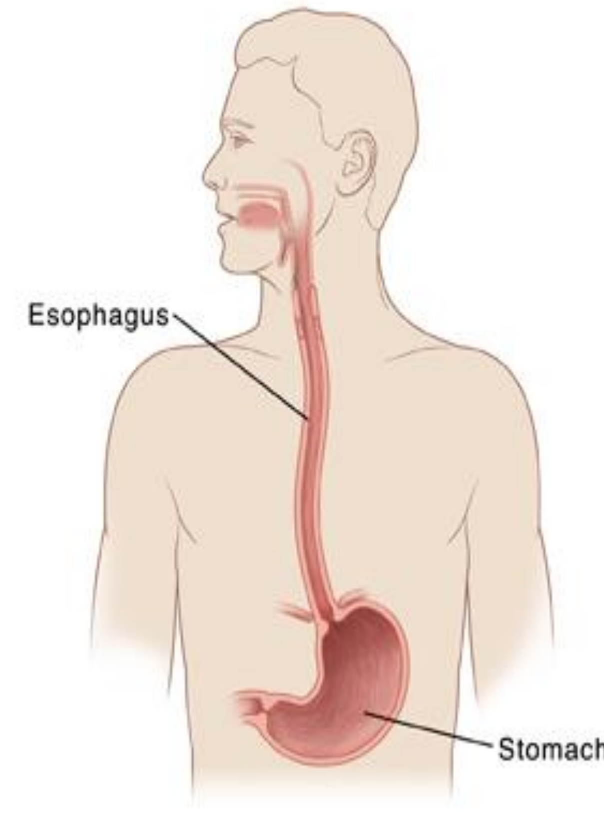


Figure 2: General picture of the esophagus

(www.uofmmedicalcenter.org)

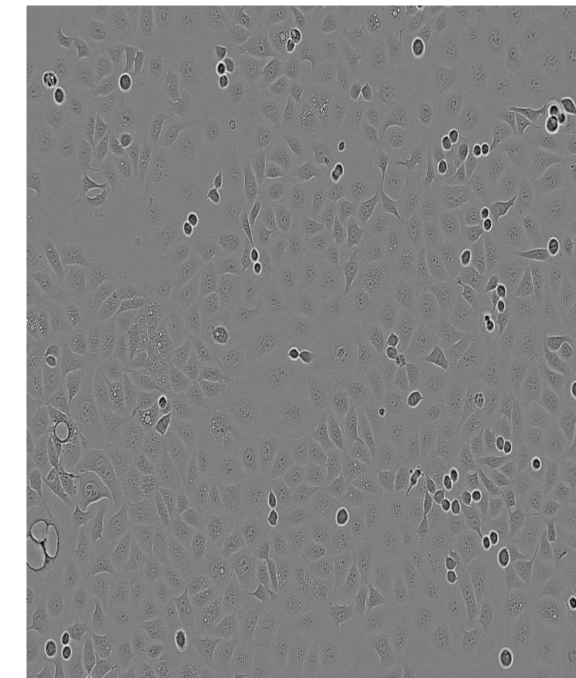


Figure 3: KYSE-30 esophageal cancer cells 48h post seeding. (www.phe-culturecollections.org.uk)

RESULTS:

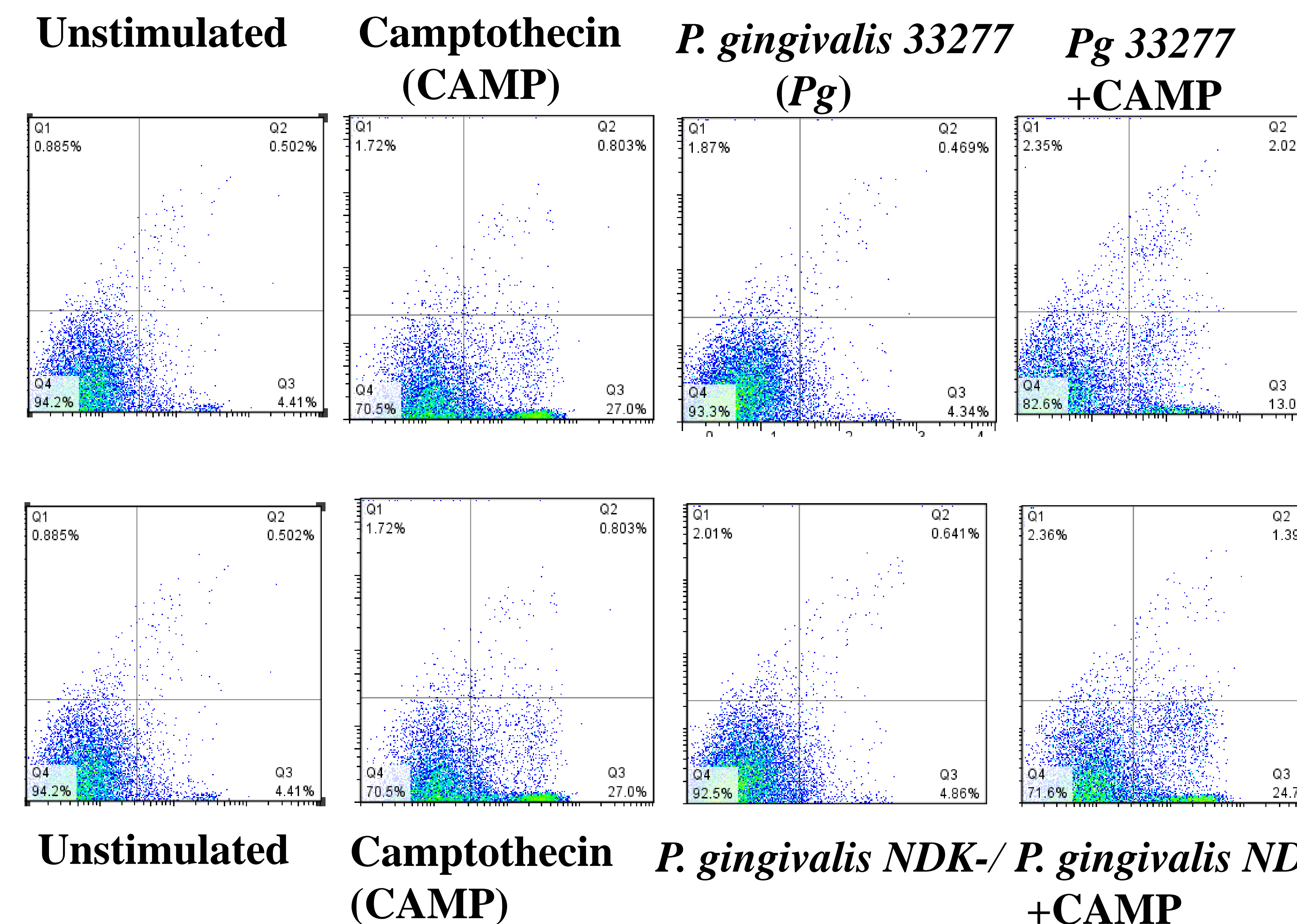


Figure 4: Anti-apoptosis effects of *P. gingivalis* on the well-differentiated esophageal cancer cell line, KYSE-30.

CONCLUSIONS:

- Infection of *P. gingivalis* 33277 confers resistance to Camptothecin-induced apoptosis in the human esophageal cancer cell line KYSE-30.
- *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.

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A Multi-Organ Study Using Microwave: A Comparison of the Solero system to the Sulis V pMTA and the NeuWave Certus 140 systems

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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 pre determined 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

Test Articles

Figure 1A. NeuWave Certus 140 Microwave Ablation System

Figure1B. AngioDynamics Sulis V Microwave Ablation System

Figure 1C. AngioDynamics Solero Microwave Ablation System

Figuer 2A. Day 1 Ablation Surgery

Dr. Martin performing microwave ablation on liver

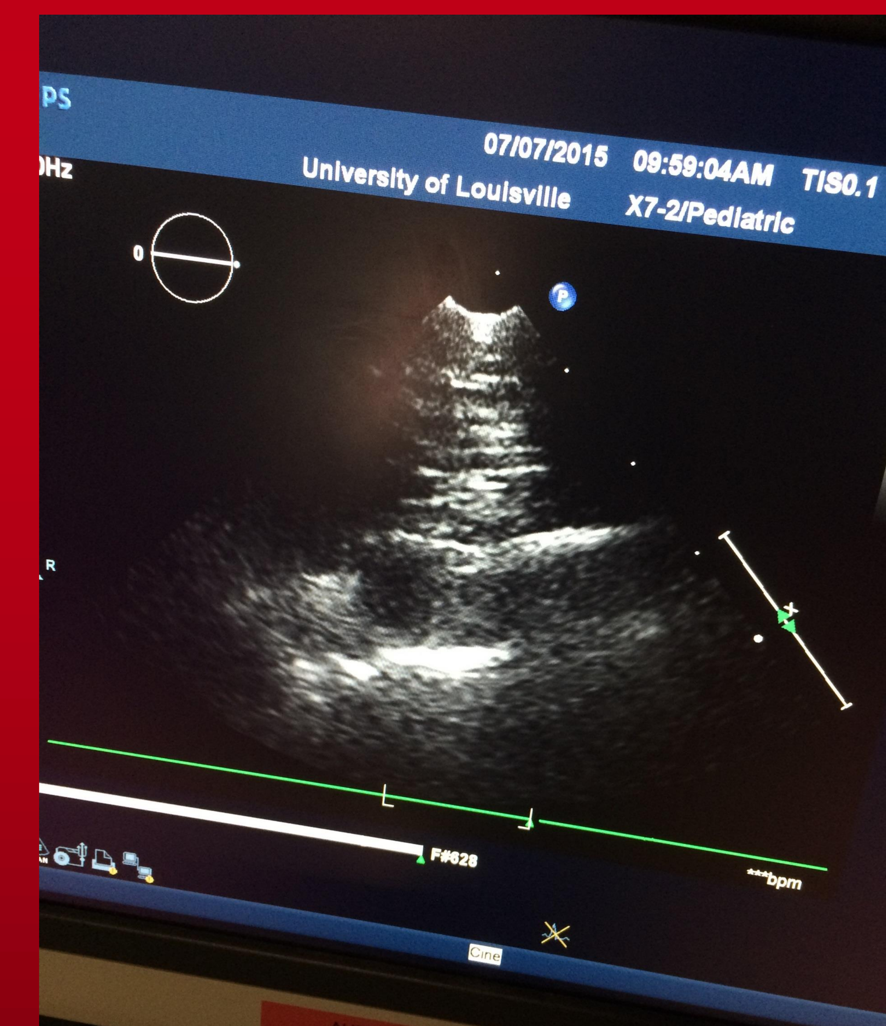

Figure 2B. Day 1 Ablation Surgery

Ultrsound Image of a kidney ablation

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.

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

Figure 3. Day 28 Necropsy

Extracted organs from Pig 10 (65668)

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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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, given 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).

Patients who presented to a Multidisciplinary Clinic with a primary HNC (N=98) completed the Distress Thermometer (DT) and Hospital Anxiety and Depression Scale (HADS). Albumin, hemoglobin, AST, and ALT values, weight change during treatment, and two-year survival data were gathered from medical records. Psychometric scores and biomarkers were entered separately as predictors, with weight loss, DFS and OS entered as outcomes in hierarchical and Cox regressions.

Patients were mostly male (75.5%), averaging 59 years of age, diagnosed with oropharyngeal (33.7%), laryngeal (17.3%), or oral (10.2%) cancers. Many reported clinically significant anxiety (42%) and/or depressive symptoms (33%). The vast majority of patients demonstrated biomarker levels within normal ranges, and 65 patients demonstrated weight loss averaging 3.6 kg. Anxiety, depressive symptoms, and malnutrition biomarkers did not relate to weight change over the course of treatment. After adjusting for age, stage, site, and treatment, anxiety was associated with poorer DFS (HR=1.124, 95% CI=1.005-1.258, p=.041), depressive symptoms were associated with poorer OS (HR=1.109, 95% CI=1.012-1.216, p=.027), and lower pretreatment hemoglobin was associated with poorer OS for males and females (HR=.740, 95%CI=.561-.977, p=.033).

Depressive symptoms are associated with a greater likelihood of poorer short-term (treatment interruption and incomplete response) and long-term (OS) outcomes in this sample of HNC patients. 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

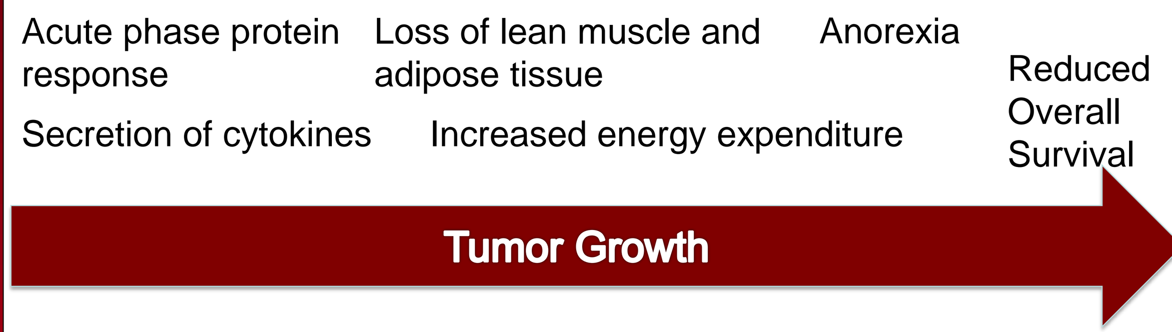
Cancers of the head and neck (HNC) account for approximately 5% of all malignancies.[1] HNCs are associated with significant distress, anxiety, and depression.[2] The extent of emotional distress may be associated with disease characteristics[3], as tumors located on a patient's face or mouth may interfere 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 asthenia. At diagnosis, 35%-60% of HNC patients are malnourished due to obstruction of intake or anorexia.[5] Cachexia may also have systemic effects that influence tumor growth and response to treatment, including alteration of immune function.[4] Commonly measured serum biomarkers, including albumin, AST, ALT and hemoglobin, serve as indicators of a patient's systemic nutritional status.[5,6]

The impact of cachexia may be significant, potentially increasing risk for poorer disease-free and overall survival.[5] Indeed, cachexia has been related to 20% of HNC-related deaths.[1,4] Early detection of cachexia risk factors may therefore help prevent poorer long-term outcomes. Psychosocial stressors and biomarkers of cachexia should therefore be evaluated at or near the time of diagnosis. Early detection may allow more opportunity for psychological and/or 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).

Methods & Results

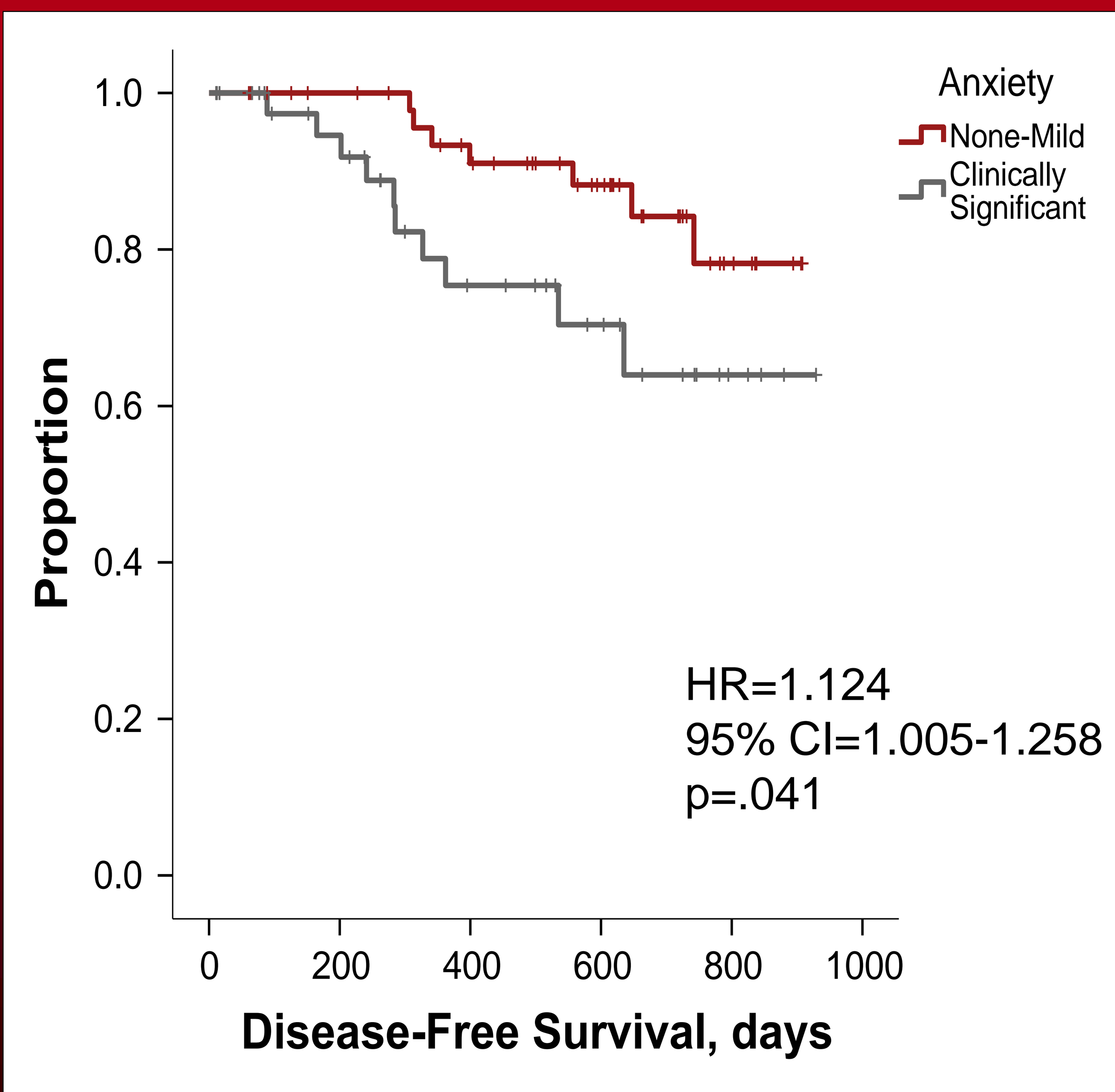


Psychometric	Description	Score Range
Distress Thermometer[7]	Patients circle a number on the thermometer that best describes the amount of distress that they have experienced in the past week.	0-4 → low or controlled 5-7 → clinically significant 8-10 → clinically significant with poor coping
Hospital Anxiety and Depression Score[8]	Assesses level of anxiety and depressive symptoms for the past week among medical populations.	0-7 → none or mild 8-21 → clinically significant

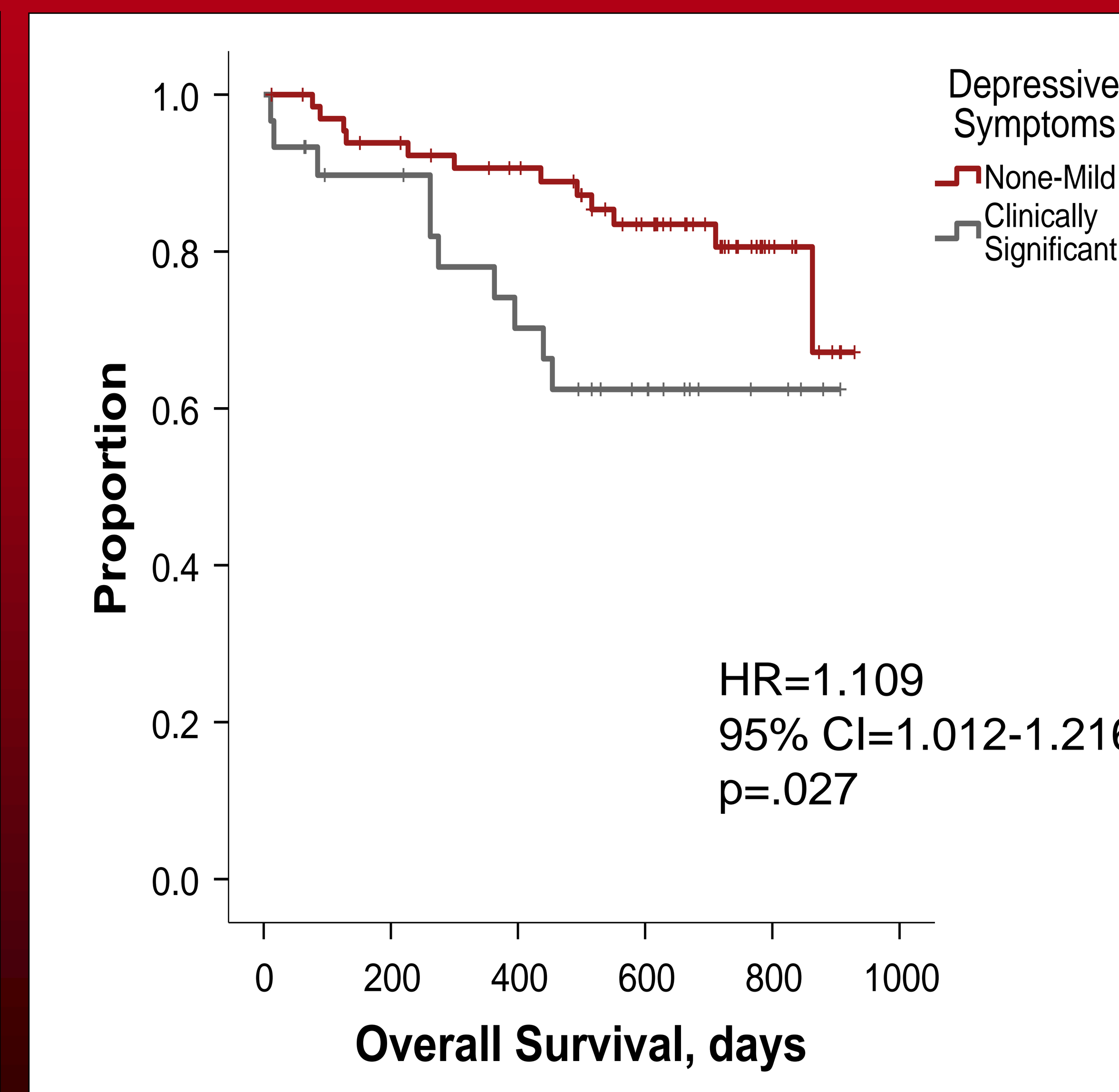
Biomarker	Normal Range	Description	Indications
Albumin	3.5-5.0 g/dL Hypoalbuminemia: <3.5 g/dL	Indicates visceral protein status and inflammation.	Low levels indicate not receiving or absorbing enough nutrients.
Hemoglobin	M 13.8-17.2 g/dL F 12.1- 15.1 g/dL Anemia: M<13.5, F<12.0	A measure of oxygen concentration in red blood cells.	Decreased hemoglobin can indicate nutritional deficiencies.
AST	10-34 IU/L	A liver enzyme.	High levels indicate liver damage or injury.
ALT	10-40 UI/L	A liver enzyme.	High levels indicate liver damage or injury.

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.

	Mean	SD	N	%
Male gender			74	75.5
Age at diagnosis	59.65	12.51		
Psychometrics				
Distress Thermometer	4.81	3.30		
Anxiety (HADS-A)	7.74	5.21		
Depressive Symptoms (HADS-D)	5.74	5.02		
Biomarkers				
Serum Albumin*	3.972	0.465		
Hypoalbuminemia			5	5.1
Serum Hemoglobin (male)	13.505	1.826		
Anemia (male)			5	6.8
Serum Hemoglobin (female)	12.270	1.422		
Anemia (female)			3	12.5
Serum AST	31.36	13.91		
Serum ALT	34.62	12.66		
Outcomes				
Weight Loss, kg	-3.62	5.18	65	66.3
Cachexia			34	27.6
Disease-Free Survival, days	495.48	261.68	17	17.3
Overall Survival, days	553.01	257.42	22	22.4



Anxiety symptoms were associated with disease-free survival



Depressive symptoms were associated with overall survival

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)[9] 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=.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.[10]

Acknowledgements

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

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Department of Pharmacology and Toxicology[1] and James Graham Brown Cancer Center[2]

INTRODUCTION

Prostate Cancer as a Public Health Problem

❖Despite improvements in the early detection of prostate cancer (PCA) and treatment strategies, men with metastatic disease have a 72% decrease in their 5-year survival rate after diagnosis.

❖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

❖Micro-RNAs (miRNA), short non-coding single stranded RNAs, may serve as effective tools to improve cancer diagnostic, prognostic, clinical management, and prevention strategies.

❖miRNAs function as oncogenes or tumor suppressors that regulate the expression of genes involved in cell growth, apoptosis, differentiation, metastasis and angiogenesis.

❖Preliminary data in our lab suggest miR-21 was over expressed in the serum collected European--American men diagnosed with prostate cancer relative to disease-free individuals.

❖The over-expression of oncomiRs. 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.

❖It has anti-oxidative, anti-inflammatory, anti-cancer properties

❖Previous studies reveal quercetin inhibits cell invasion, migration, and proliferation in PC-3 prostate cancer cell lines as well as modulates expression of DNA repair, extracellular matrix degradation and tumor invasion, angiogenesis, apoptosis, and cell cycle genes.

❖Recent studies and clinical trials suggest quercetin has activity against tumor growth.

❖A few *in vivo* 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 Caucasian metastatic line.

❖ Assess the impact of quercetin treatment on cell proliferation and cell 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 oncogenic miRNA-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 oncomiRs and treatment of pre and metastatic prostate cancer.

RESULTS

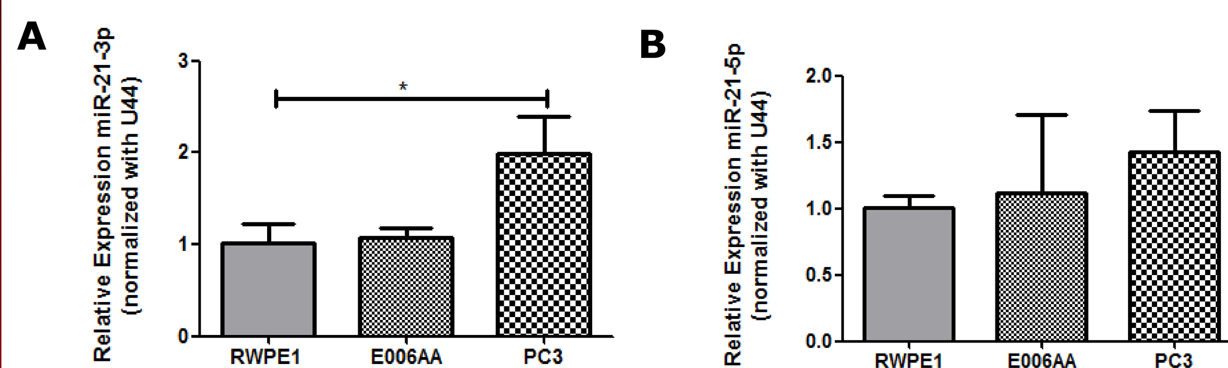


Figure 1. Relative expression levels of mir 21-3P(A) and mir 21-5P(B) were found via qPCR for RWPE1, E006AA and PC-3 cell lines Statistical significance was determined using the Unpaired T-test (* p = 0.011).

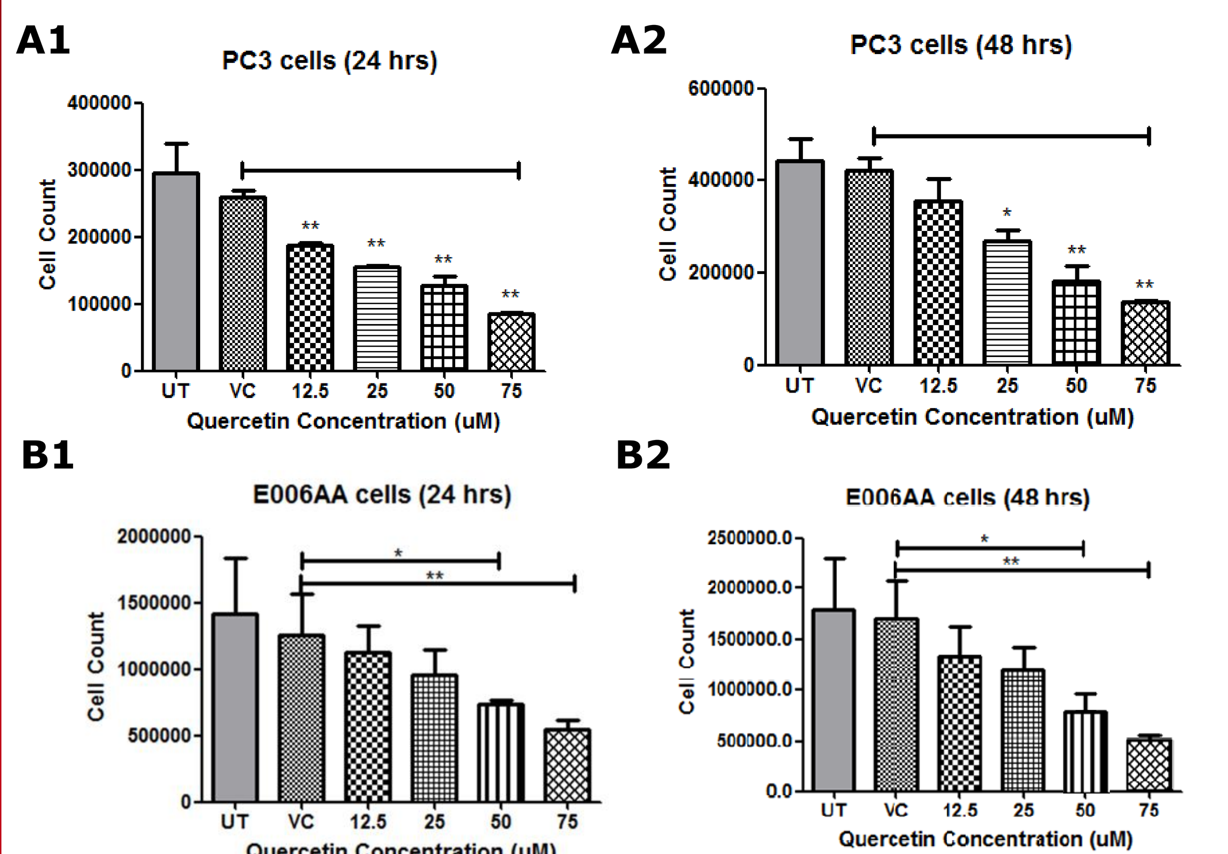


Figure 2. PC3(A) and E006AA(B) cells were treated with quercetin (12.5-75μM) compared with vehicle control (0.0375% DMSO) and untreated for 24 and 48 hrs. Trypan Blue assay was utilized for cell counting. Following quercetin treatments an EC50 was established via GraphPad Prism. Statistical significance was determined using the one-way ANOVA (Non-parametric) and Unpaired T-test (*p ≤ 0.05 ** p ≤ 0.0096.

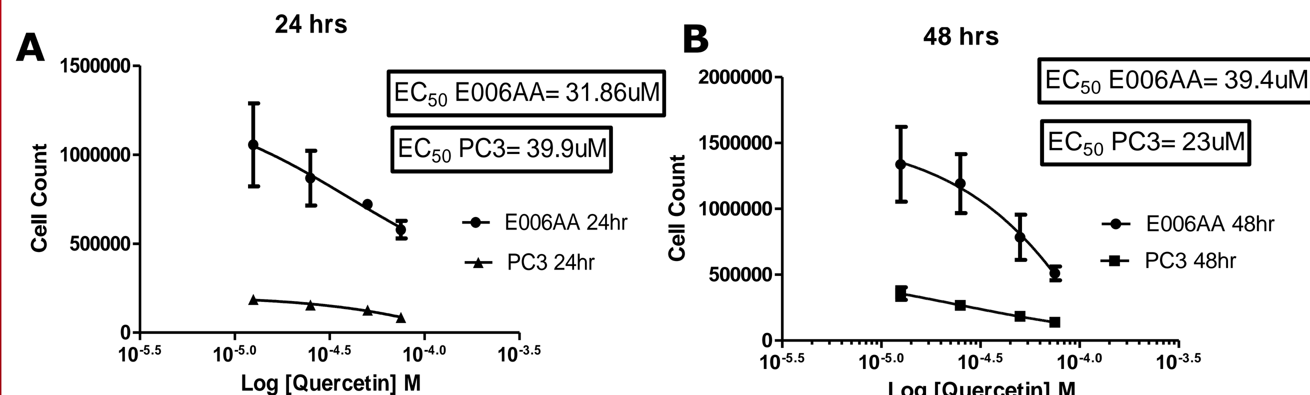
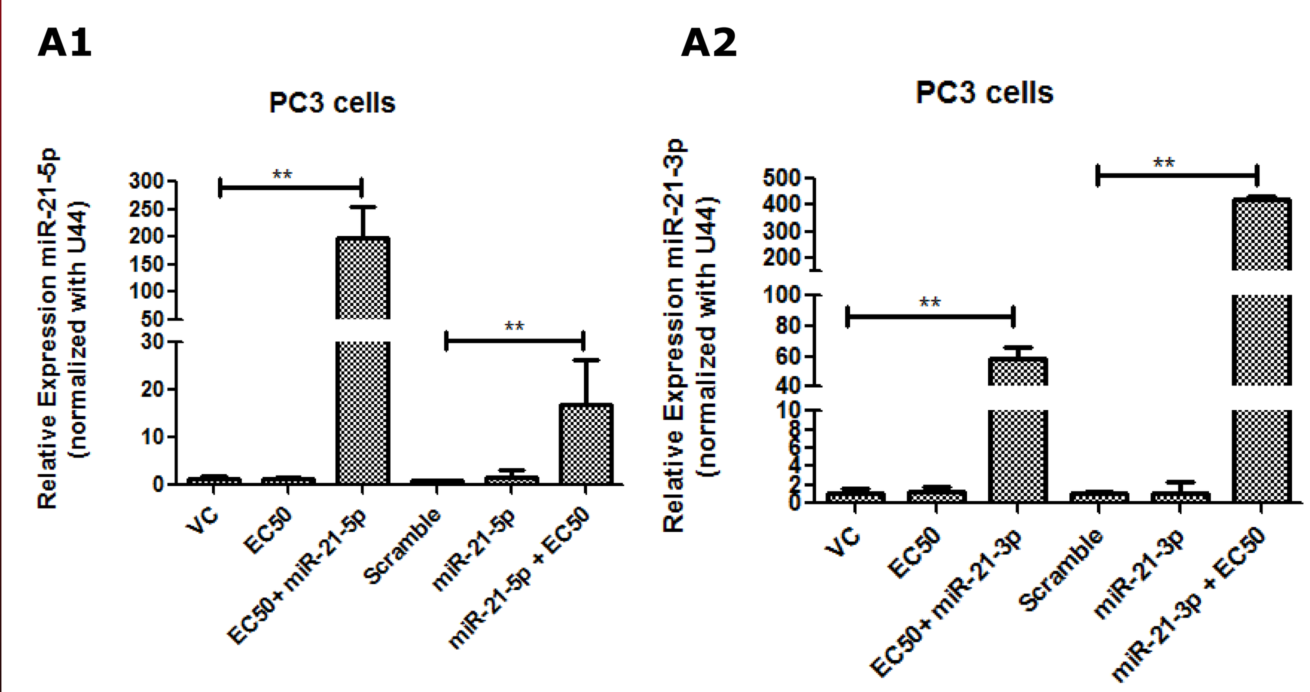


Figure 3. Effective concentration (EC50) was calculated at 24 and 48 hrs for PC3(A) and E006AA(B) cells using the Trypan blue assay. Cells were treated with (12.5-75μM) quercetin and vehicle control (0.0375% DMSO). After a 24hr quercetin treatment, 31.9-39.9μM served as the EC50 for the E006AA and PC3 cell lines. After 48hrs, EC50 was 39.4μM and 23μM for E006AA cells and PC3 cells, respectively.



RESULTS

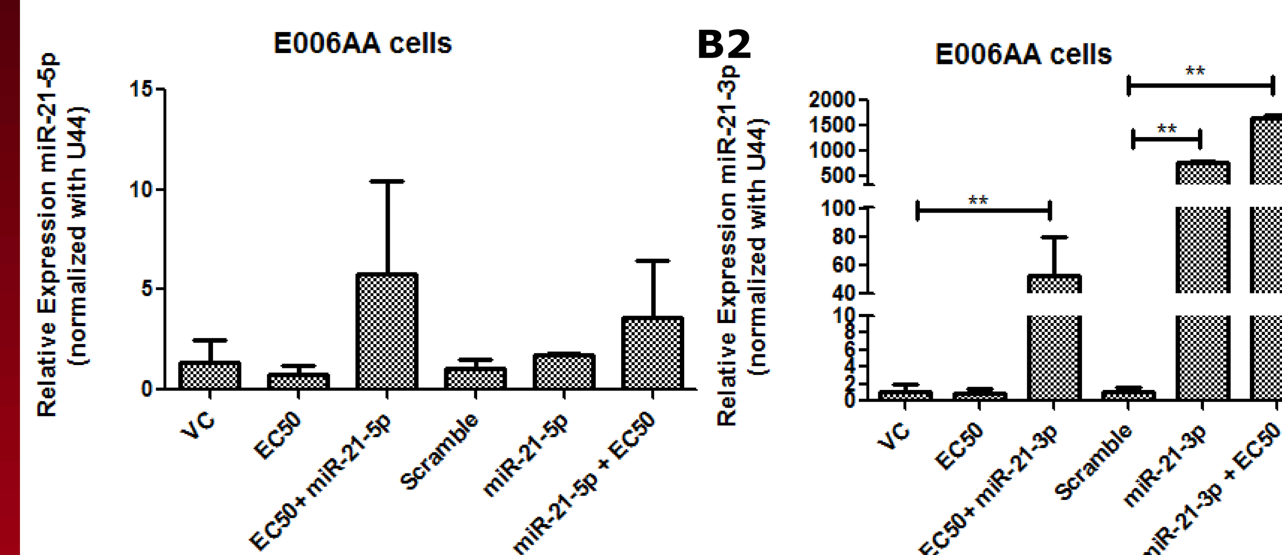
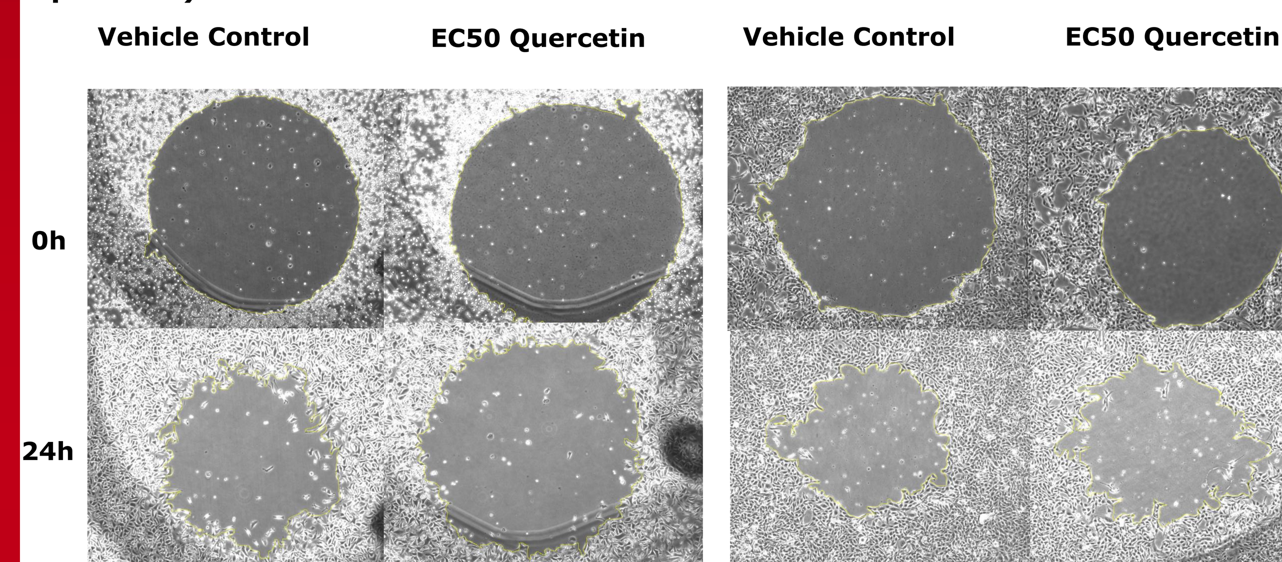


Figure 4. The impact of quercetin on mir21-3P and mir21-5P levels was assessed using qRT-PCR in cell lines ectopically expressed with miR-21. PC3 (A) and E006AA (B) were treated with vehicle control, quercetin EC50, scramble, miR-21 mimic (-3p and -5p), and combination of mimic and quercetin EC50 for 24 hrs. Total RNA was extracted from prostate cancer cell lines using the Mirvana miRNA Isolation kit. Relative miR-21 levels were normalized with U44. Statistical significance was determined using one-way ANOVA and Unpaired T-test (** and * p ≤ 0.05).



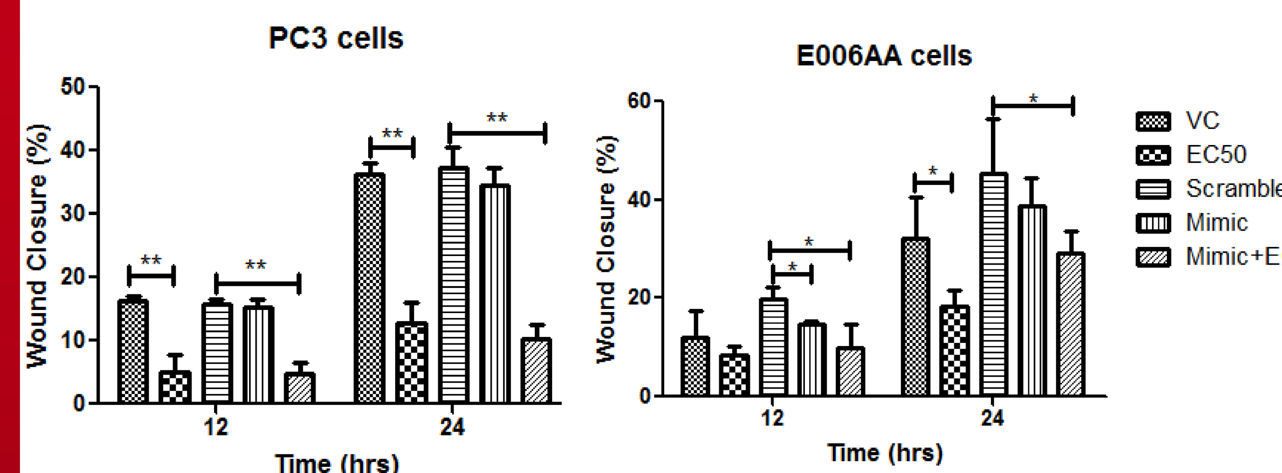


Figure 5. The impact of quercetin on cell migration in cell lines transiently transfected with miR-21 was assessed using a modified wound healing assay. PC3 and E006AA cell lines were treated with vehicle control, quercetin EC50, scramble, mimic miR-21-3p and combination of mimic and quercetin EC50. Wound healing assay was performed and photographed under phase-contrast microscopy (4x). Statistical significance was determined using one-way ANOVA and Unpaired T-test (**p ≤ 0.002, * p ≤ 0.05).

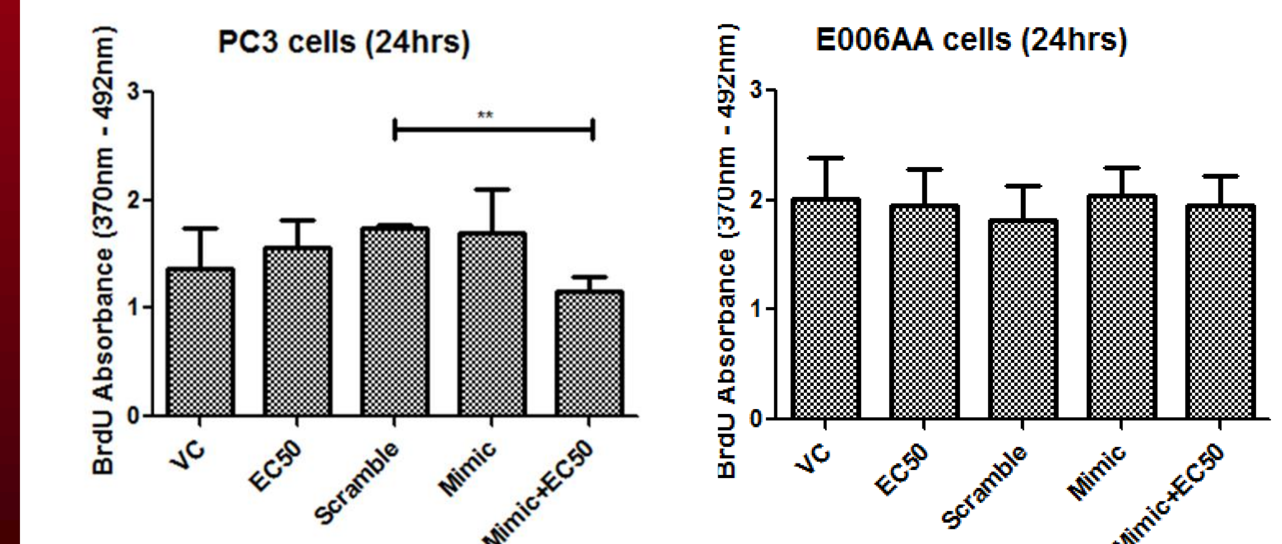


Figure 6. The impact of quercetin on cell proliferation in cell lines transiently transfected with miR-21 was assessed using the BrDU assay. PC3 and E006AA cells were plated in 96-well plates at optimal cell density (2,000 cells/well). Cells were treated with vehicle control, quercetin EC50, scramble, mimic miR-21-3p and combination of mimic and quercetin EC50. Statistical significance was determined using one-way ANOVA and unpaired T-test (** p = 0.001).

DISCUSSION & CONCLUSIONS

Baseline Levels of miR-21 in Prostate Cancer Cell Lines

❖ miR-21-3p was significantly up-regulated in PC3 cells compared to control cell line (RWPE1).

❖ However, miR-21-5p expression was not differentially expressed in E006AA and PC3 cells compared to RWPE1 cells.

Impact of Quercetin on PCA Cell Population In Vitro

❖ Relative to the vehicle control (0.0375% DMSO)
  - ➢ Cell population of PC3 cells was decreased significantly by quercetin treatment (25-75μM) within 24hr and 48hr time points
  - ➢ Cell population of E006AA cells was decreased significantly by quecertin (50-75μM) within 24hr and 48hr time points

Quercetin Effective Concentration In Vitro

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

Impact of Quercetin on Cell migration using a Wound Healing Assay

❖ A significant 18.6 % 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 PC3 cells treated with EC50 and miR-21-3p mimic.

❖ No significant differences was 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 treatments will:
  - ➢Down-regulate the expression of oncomiRs or up-regulate the expression of tumor suppressing miRs 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-2b).
  - ➢Reduce tumor size, tumor number, or metastasis using animal models

❖ Modify miRNA targets and corresponding proteins using PCA or normal epithelial cell lines transfected with miRNA mimics or inhibitors

❖ Evaluate whether a quercetin metabolite or quercetin analogs will have a more pronounced effect on modulating the expression of human miRs and/or PCA phenotype

❖ Assess whether quercetin treatment combined with conventional drugs may help increase survival rates among pre- or metastatic PCA patients

ACKNOWLEDGEMENTS

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

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

❖ "Our Highest Potential" Endowed Chair in Cancer Research Endowment to LRK.

Temozolomide Enhances Breast Cancer Virotherapy Regardless of Estrogen Receptor Status

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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-cytopathic 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.

To the best of our knowledge, this is the first time that a chemotherapeutic drug designed to treat melanoma and glioblastoma is used to enhance the oncolytic Ad mediated-breast cancer killing effect.

Results

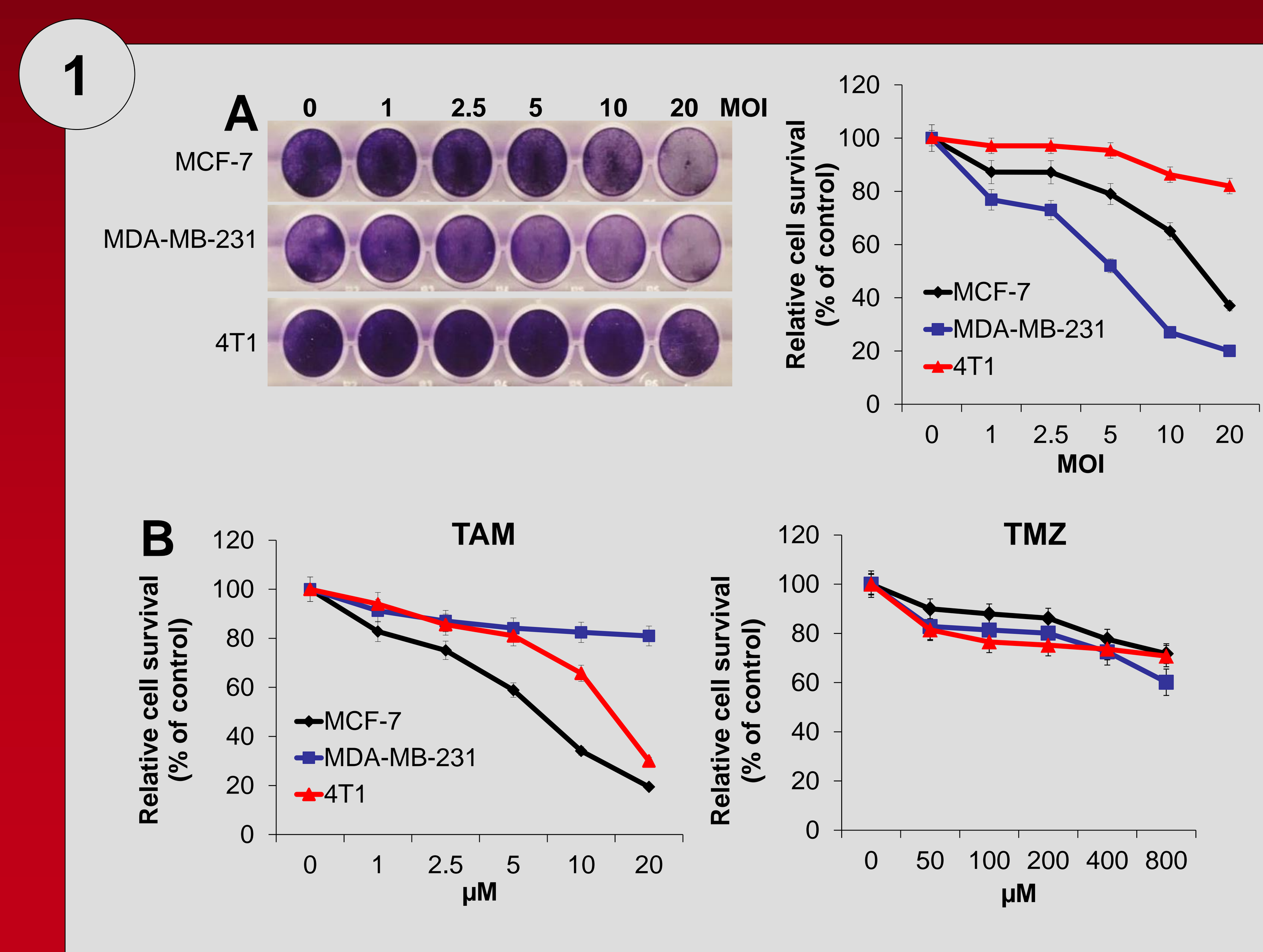

Fig. 1 A) Crystal violet staining to evaluate the Adhz60 cytopathic effect in BC cells. **B) MTT assay** to assess the cytotoxic effect of TAM or TMZ in BC cells. (72h post treatment)

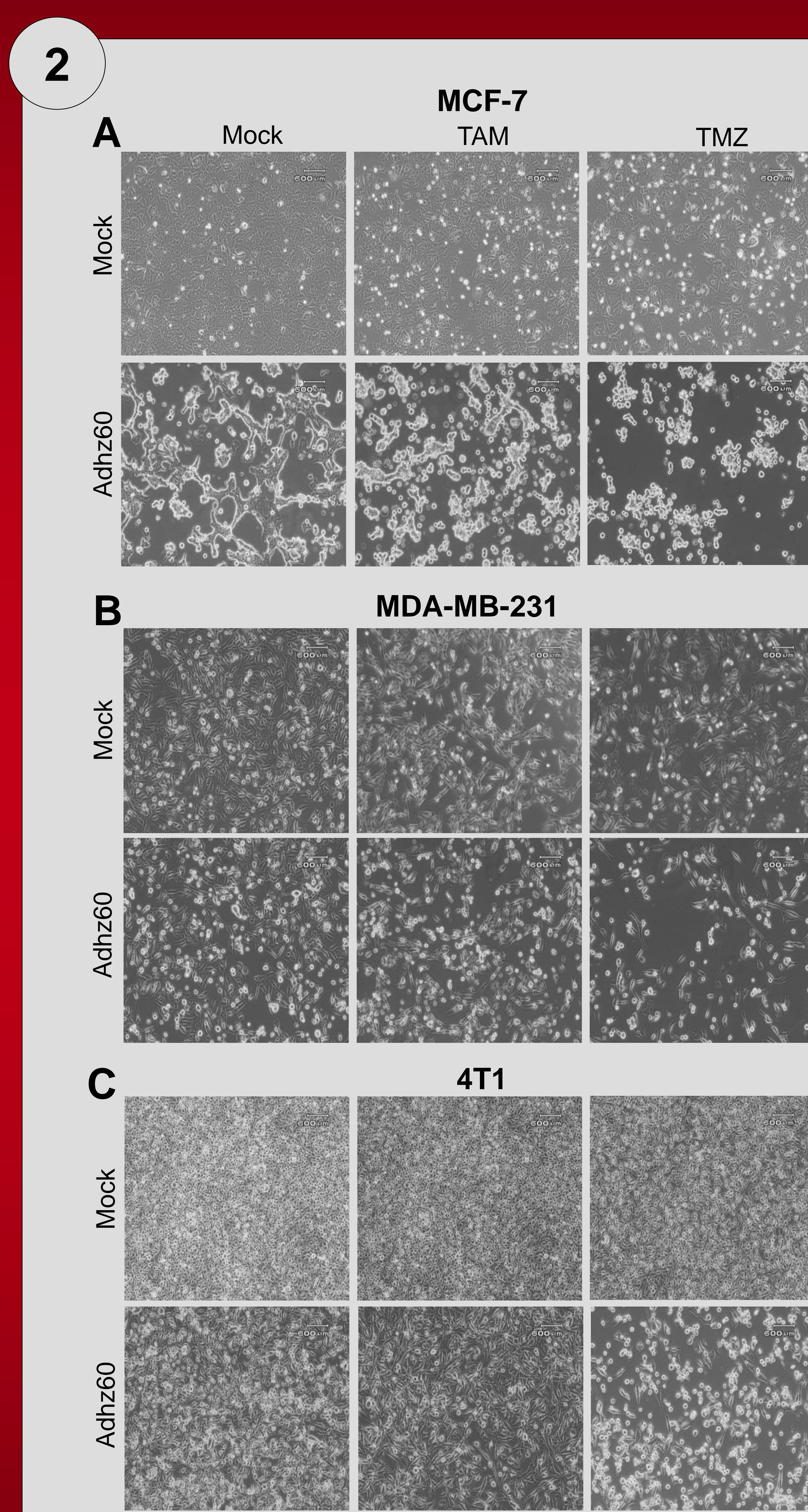

Fig. 2. Evaluation of the Adhz60 mediated-cytopathic effect alone or in combination with TAM or TMZ in BC cells. (72h post treatment)

A) MCF-7 ER-positive

B) MDA-MB-231 ER-negative

C) 4T1 murine ER-positive

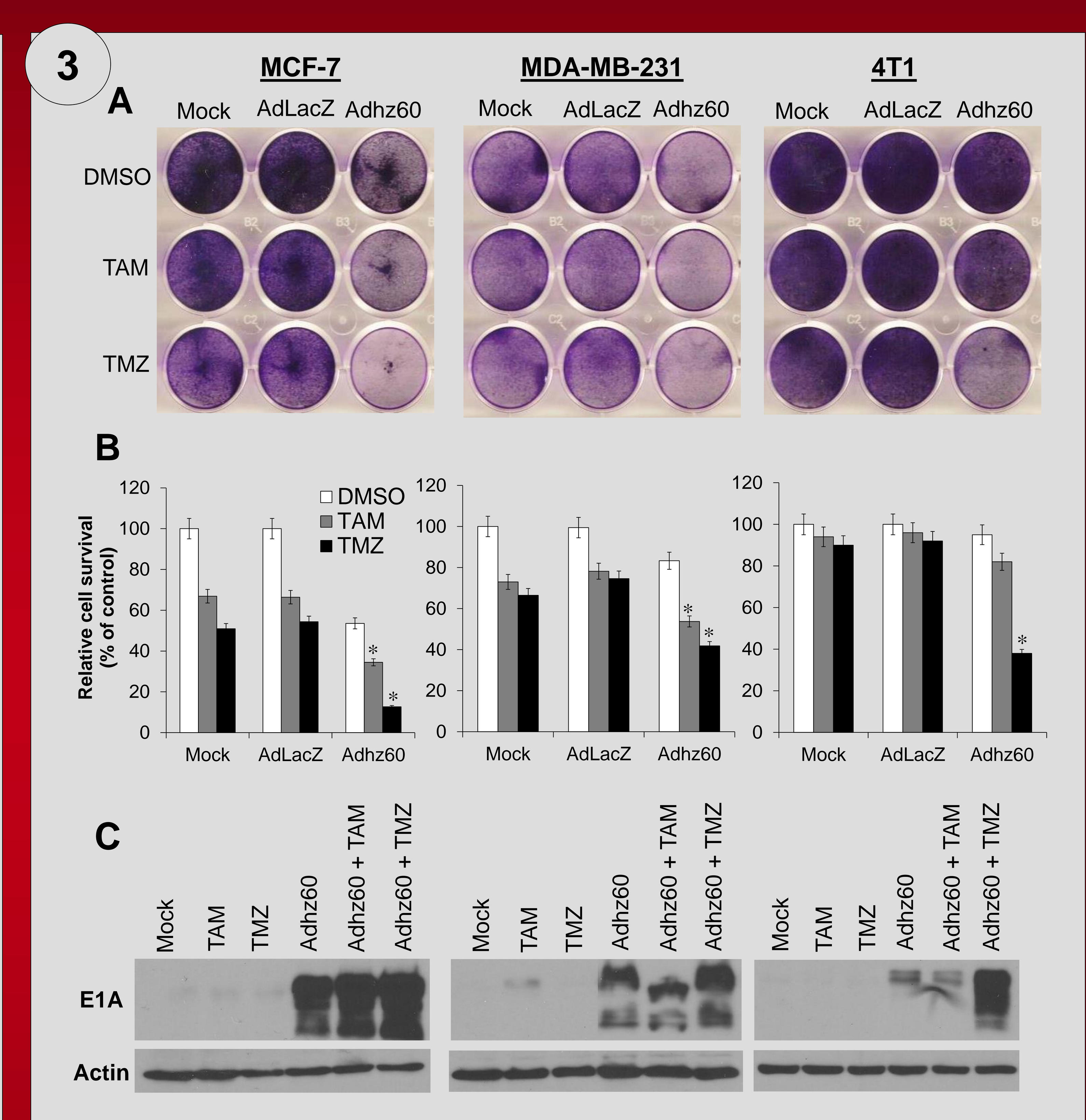

Fig. 3. Evaluation of the combined therapy on breast cancer cells. A) Crystal violet assay to evaluate the cytotoxic activity of the combined therapy with Adhz60 and TAM or TMZ; **B)** Cell viability was calculated by measuring the absorbance of solubilized dye at 590 nm. Each point represents the mean of three independent experiments ± standard deviation (SD; *bars*); **C)** WB to assess the adenovirus E1A expression. (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.

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.

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

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Introduction

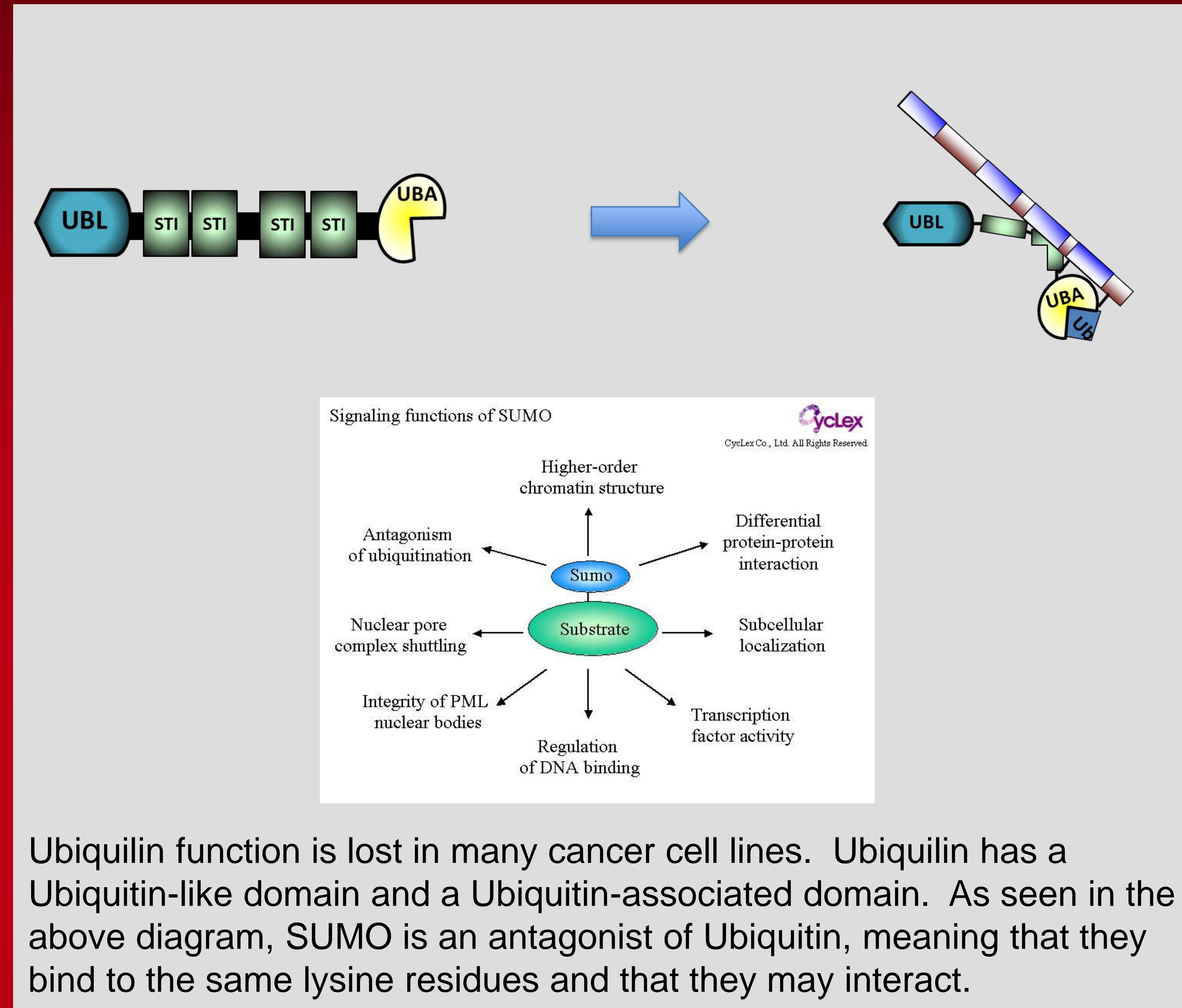
Post-translational modifications often dictate the fate of a newly synthesized protein. These modifications, or lack thereof, can result in an activation or deactivation of a protein, and many other functions. One of these modifications are a family of proteins called small Ubiquitin-like modifiers (SUMO). 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.

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.

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.

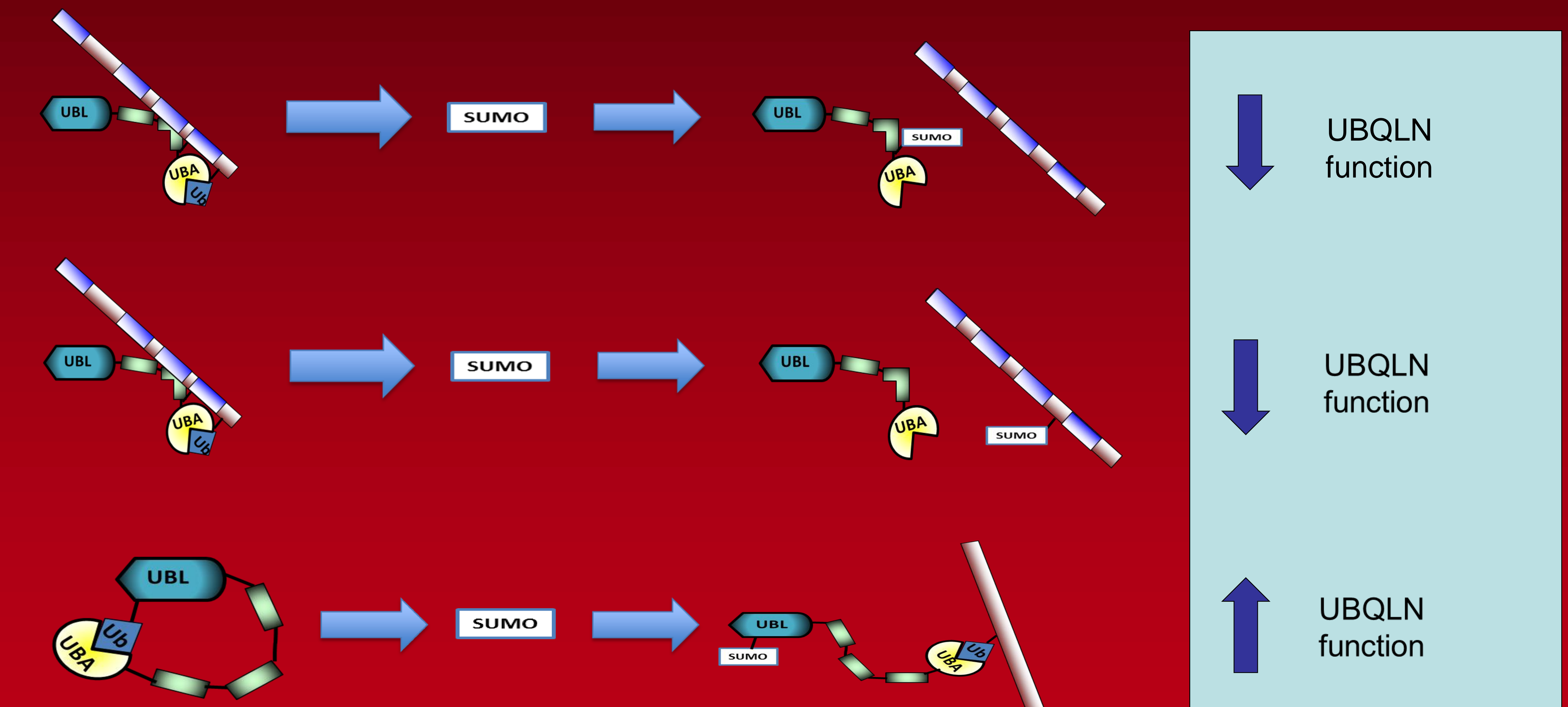
Methods

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.

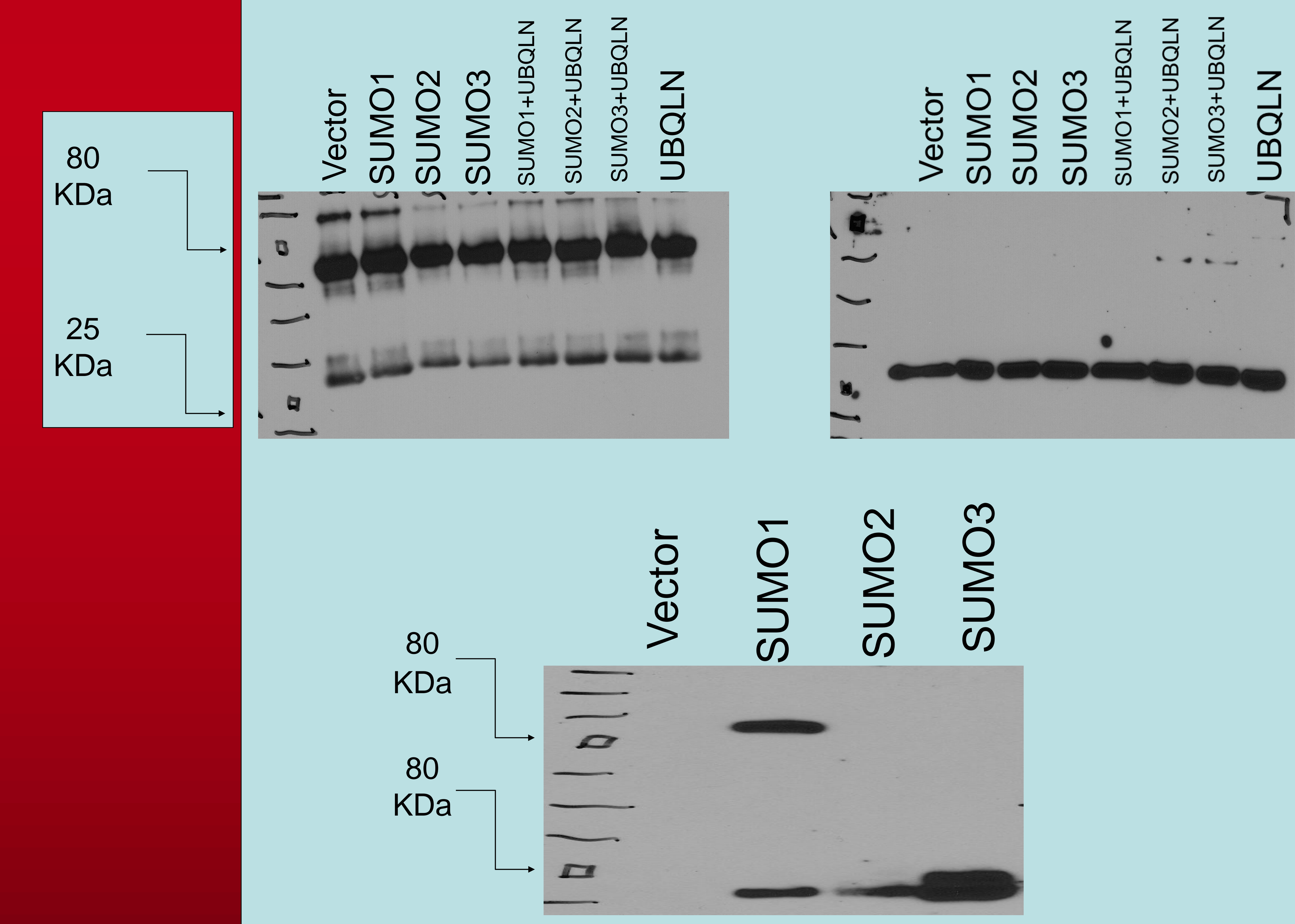
Conclusion/Future work

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.

Models



Results



Acknowledgements

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