Elizabethtown College
JayScholar

Biology: Student Scholarship & Creative Works | Biology

Spring 2016

Cannabinoid Receptor 2 Expression and its Role in Echinacea Signaling

Nathaniel Hunter Williams
Elizabethtown College, williamsn@etown.edu

Follow this and additional works at: https://jayscholar.etown.edu/biostu

Part of the Biology Commons

Recommended Citation

Williams, Nathaniel Hunter, "Cannabinoid Receptor 2 Expression and its Role in Echinacea Signaling" (2016). *Biology: Student Scholarship & Creative Works*. 3.
https://jayscholar.etown.edu/biostu/3

This Student Research Paper is brought to you for free and open access by the Biology at JayScholar. It has been accepted for inclusion in Biology: Student Scholarship & Creative Works by an authorized administrator of JayScholar. For more information, please contact kralls@etown.edu.

Cannabinoid Receptor 2 Expression and its Role in Echinacea Signaling

Nathaniel Hunter Williams

Honors In the Discipline Senior Thesis

05/06//2016

Dr. Jane Cavender

Elizabethtown College

Abstract

Echinacea, a commonly used herbal supplement, is publicized for its antioxidant and immunostimulatory properties. It has been used to treat the common cold and upper respiratory infections but has more recently been touted as a potential target for chemotherapeutic treatment. Echinacea is believed to work through the cannabinoid receptor 2 (CBR2), a G-protein coupled receptor primarily expressed in inflammatory and immune-competent cells. Activation of CBR2 is believed to induce the MAPK pathway as well as increase levels of cAMP. Recent research has shown tumor expression of CBR2 which has been correlated to a decreased patient prognosis though the cellular pathway has not yet been elucidated. This study was designed to gain a clearer understanding of the relationship between Echinacea, the cell cycle and CBR2. To assess the effect of Echinacea on cell growth, HeLa and human diploid fibroblast (HDF) cells were seeded and treated for 24 hours with 0, 25, 50 or 100 ug/ml of the water solubilized extract. The effect on cyclin A, cyclin E levels was assessed using the luciferase reporter assay. Additionally the presence of the CBR2 receptor was determined by using immunofluorescence and immunoblot analysis. Our results show that cyclin activity appeared to be positively correlated to tumor cell proliferation following increased treatments of Echinacea. Expression of CBR2 was visualized using immunofluorescence and accumulated protein levels were assessed by imunoblot analysis. CBR2 was detected in both cell lines at varying levels. With these results it is hypothesized that the Echinacea may be activating CBR2 and inducing the MAPK pathway. In turn, the MAPK pathway activated the cyclins and caused increased cell proliferation.

Introduction

Echinacea

A relative of the daisy, *Echinacea purpurea* is a species of Echinacea that is commonly used as an herbal supplement for its antioxidant, anti-inflammatory, and immunostimulatory properties (Wang et al, 2006). Though not certified by the FDA, Echinacea supplements can be found in both pill and oil form and are used in a variety of applications (Approved Drug Products with Therapeutic Equivalence Evaluations, 2005; Echinacea, 2012). The supplements are regularly used as a remedy for upper respiratory tract infections, the common cold, as well as both bacterial and viral infections (Raduner et al, 2006). Despite its widespread medicinal use, only a few studies have supported the clinical efficacy of Echinacea (Raduner et al, 2006).

Marlies et al (2014) examined the impact of *Echinacea purpurea* treatment for the common cold and concluded that there was a possible increase in prevention. The study examined 24 clinical trials with various Echinacea preparations. The preparations differed in both species of Echinacea as well as what part of the plant was used. While some of the preparations were found to increase common cold prevention, the majority did not (Marlies et al 2014). In a review by Shah et al (2007) found there to be a decrease in the duration of the common cold in subjects who were treated with Echinacea (Shah et al, 2007).

Echinacea purpurea displays the qualities of an immune system stimulator. It acts on the immune system by primarily affecting the innate immune system. Echinacea treatment leads to both increased proliferation and activity in natural killer cells along with macrophage activation and increased phagocytic activity (Mistríková et al, 2006). The immunostimulatory properties of Echinacea have led to its widespread use as an herbal supplement with chemotherapy for patients with cancer. Over 50% of patients with cancer use herbal supplements to complement their

treatment with Echinacea being the most frequently used (Block et al, 2003). Lersch et al (1990) did investigate the clinical effectiveness of Echinacea supplementation on five patients with inoperable, advanced hepatocellular carcinomas. While the study did find modest improvements both clinically and immunologically, there was no control in the study and the improvements could not be solely linked to Echinacea as the patients were also given a variety of other immune modulators (Lersch et al, 1990). Interestingly, many of the phytochemicals of Echinacea are classified heptatoxic (Miller et al, 1998).

Contradictory to the popularity of the use of Echinacea as a complement to chemotherapy, Cichello et al (2015) revealed an increased proliferation of several cancers *in vitro* following Echinacea treatment. HeLa cells and QBE-939 cells both displayed increased proliferation raising concerns about the possibility of Echinacea enhancing tumorgenesis (Cichello et al 2015). With Echinacea containing numerous compounds, there is little knowledge on which chemicals are mechanistically responsible for stimulating cell proliferation.

The pharmacological compounds found in Echinacea can be separated into three distinct categories: alkylamides and polyenynes, caffeic acid derivatives, and polysaccharides (Mistríková et al, 2006). Several studies have investigated the cellular mechanisms of the various compounds. Huntimer et al (2006) treated HeLa cells with chicoric acid and cynarine, both phenolic compounds in Echinacea, and found increased cell growth (Huntimer et al, 2006). Gurtsch et al (2004) found alkylamides in Echinacea to upregulate TNF-alpha mRNA in primary human monocytes/macrophages presumably through interacting with the cannabinoid receptor type 2 (CBR2). Additionally, it was found that alkylamides led to increased cAMP and activated JNK and p38/MPAK kinases. Finally, it was suggested that the alkylamides activated ATF-2/CREB-1 and NF-κB downstream (Gurtsch et al, 2004). These stimulatory cellular mechanisms

are presumably modulated through the CBR2 receptor found on the immune cells. Thus, its presence appears to play a vital role in the Echinacea signaling.

CBR2 Receptor

CBR2 is a 7-pass transmembrane G-protein coupled receptor that is similar in structure to the cannabinoid receptor type 1(CBR1). CBR1 is found in high levels throughout the brain and has proven to be essential to behavior involving fear, anxiety and stress (Monory et al, 2015). CBR1 is also the target of THC, the main psychoactive constituent of cannabis (Sim-Selley et al, 2003). CBR2 has been identified on a wide array of cell types including keratinocytes, immune cells, microglia, perivascular cells, and more recently tumor cells (Romero-Sandoval et al, 2008; 2009).

The cannabinoid receptors are responsible for controlling many cell signaling events downstream. CBR1 suppresses adenylate cyclase through an alpha inhibitory (α_i) that inhibits cAMP-dependent pathways (Samson et al, 2003). Via its Gβγ subunits, CBR1 is also able to impact downstream pathways that are independent of cAMP (Samson et al, 2003). Gβγ is involved in signal transduction to phosphatidylinositol (PI) 3-kinase and mitogen-activated protein kinases (Samson et al, 2003). CBR2 has been found to induce different downstream events. Gertsch et al (2004), displayed that CBR2 agonists derived from Echinacea caused an increase in cAMP levels which were eliminated upon treatment with pertussis toxin (Gertsch et al, 2004). This suggests that the CBR2 activation results in increases in cAMP levels in x cells. Transcriptionally, the cannabinoid receptors can regulate both CREB and NF-κB expression (Samson et al, 2003). Romero-Sandoval et al (2009) concluded that CBR2 agonists cause inhibition of the extracellular signal-regulated kinase (ERK) through mitogen-activated protein

kinase-phosphatase (MAPK) induction. Specifically, the expression of MKP-3 is increased resulting in a change from a pro-inflammatory microglial phenotype to an anti-inflammatory microglial phenotype (Romero-Sandoval et al, 2009).

With the recent discovery of CBR2 expression in certain tumor cells, CBR2 is now being investigated as a potential therapeutic target. Initial studies revealed that CBR2 agonists resulted in tumor cell growth inhibition; however, Perez-Gomez et al (2015) established that activation of Human Epidermal Growth Factor 2 (HER2) signaling via CBR2 correlated strongly with tumor progression and poor patient prognosis (Perez-Gomez, 2015). Interestingly enough, CBR2 is not expressed in all tumors and even shows varying expression in tumors of the same cell type such as non-small cell lung cancer (NSCLC). In patients suffering from NSCLC, only 55 percent were found to possess tumors expressing CBR2 according to IHC analysis. It is known that NSCLC results in the overexpression of epidermal growth factor receptor (EGFR), which is thought to stimulate both cell proliferation and survival. Preet et al (2011) showed metastasis in NSCLC cells treated with synthetic cannabinoids. With these results it is hypothesized that CBR2 activation altered intracellular signaling pathways linked to EGFR (Preet et al, 2011).

In this study investigated the effect of Echinacea on cell growth, cyclin expression, and separately discern the expression of CBR2 in five different cell lines: HeLa, 293T, HaCAT, HDF, and CV1. It was found that Echinacea increased HeLa cell growth and that all cell lines expressed CBR2, though at different levels and variable staining pattern. Cyclin E and A activity was examined to ascertain a possible cellular mechanism to explain the increased HeLa cell growth upon increasing treatments of Echinacea. With cyclin levels mirroring the increase in proliferation upon increased Echinacea treatment, it was hypothesized that Echinacea is promoting tumor cell proliferation through the MAPK pathway.

Materials and Methods

Cells and Cell lines.

Monkey kidney fibroblast (CV1), human keratinocyte (HaCAT), human diploid fibroblasts (HDF), HeLa (cervical carcinoma), and 293T (human embryonic expressing SV40 T antigen) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 0.03% glutamine, containing 0.225% NaHCO3, 100 µg/ml streptomycin, and 100 U/ml penicillin and grown at 37˚C with 5% CO2**.** *Cell lines were provided by Dr. M.J. Tevethia (PSU College of Medicine) and Kathleen Rundell (NorthwwesternUniv)*

Immunofluorescence.

Cells were seeded in duplicate at $6x10^5$ cells per 60 mm dish containing 4 coverslips. When cells were 80% confluent, (approximately 24 hours) media was removed by aspiration and monolayers were washed 2 times with PBS (phosphate buffered saline). Cells were fixed to coverslips by incubation with a 50:50 solution of methanol:acetone. After 5 minutes, fixative was removed and coverslips air-dried. Coverslips were rehydrated for 2 minutes with PBS and then transferred to a new 60mm dish and 100ul of primary antibody (1:100 dilution in phosphate buffered saline (PBS)) for the CBR2 (Santa Cruz Biotechnology), and/or SV40 T antigen (PAB901, kindly provided by Todd Schell, PSU HMC) was added. After 30 minutes at 37˚C and 5% CO2, the slips were washed 3 times with PBS. Secondary antibody (100ul, 1:200 dilution in PBS) with an attached FITC and/or rhodamine fluorochrome was added to the coverslips and incubated for 30 minutes at 37˚C. Following incubation the dish containing coverslips was flooded and aspirated 3 times with PBS. Lastly, the coverslips were washed with dH2O, air

dried and mounted with Vectashield containing DAPI. The coverslips were viewed using fluorescence microscopy.

Western Blotting.

Cells were lysed following the procedure outlined in Cavender et al 1999. Briefly, a Bradford protein assay was performed and equal protein concentrations (100ug) were loaded into 4-20% PAGE gels. After electrophoresis the proteins were transferred to nitrocellulose using the iBlot (Invitrogen). Membranes were blocked with 5% non-fat dry milk and then subjected to Western blotting with anti-cannabinoid 2 receptor antibody (Santa Cruz Biotechnology) followed by detection with the Protein A conjugated to horseradish peroxidase (1:200 dilution in phosphate buffered saline (PBS)) (Amersham). Luminescence was generated using the Pierce Super Signal reagent (Thermo) and detected using the Genome digital capture device.

Results

Upon treating the HeLa cells with varying concentrations of Echinacea, cell counts were obtained from four experiments done in triplicate in 12-well plates as seen in Figure 1. With the 0 µg/ml treatment there was an average cell count of 8.7×10^4 cells with a standard deviation of 5.4×10^4. With the 25µg/ml treatment there was an average cell count of 1.1×10^5 cells with a standard deviation of 6.9×10^4. With the 50 µg/ml treatment there was an average cell count of 1.3×10^5 cells with a standard deviation of 7.9×10^4. With the 100 µg/ml there was an average cell count of 1.4×10^5 cells with a standard deviation of 1.0×10^5. Significant increase in growth compared to the control was found in both 50µg/ml and 100 µg/ml Echinacea treatments of HeLa cells ($p<0.05$).

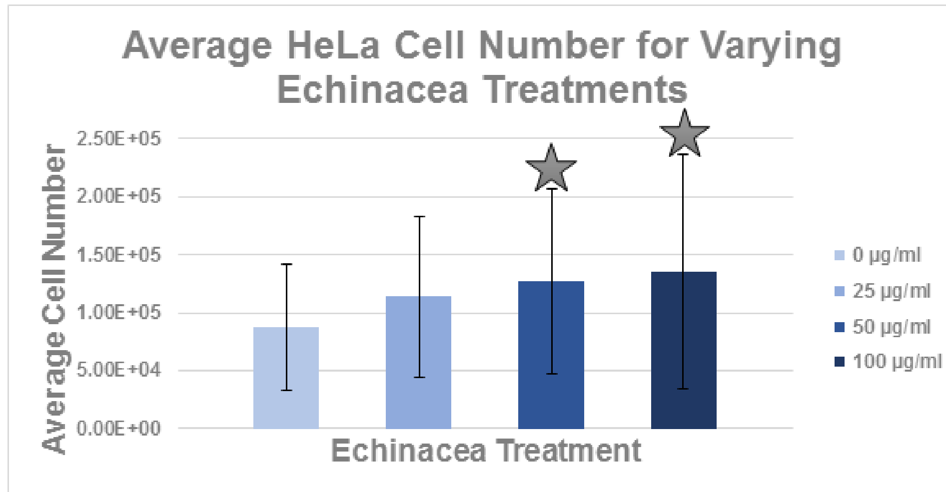

Figure 1: Displays the average HeLa cell growth of 4 experiments done in triplicate in 12-well plates following varying treatments of Echinacea. Stars denote significance with a p-value <0.05.

After noting the increased cell proliferation following increased treatment of Echinacea, HeLa cells were treated with CBR2 antibody and placed under immunofluorescence to assess for CBR2 expression (Figure 2A). The HeLa cells showed definitive CBR2 expression. It was then hypothesized that CBR2 expression could be an artifact of cell culture and four other cell lines, 293T, HaCaT, CV1, and HDF, were examined for CBR2 expression. As seen in Figure 2B-E, 293T, HaCAT, and CV1 cells all appeared to be expressing CBR2 with possible expression in HDF cells.

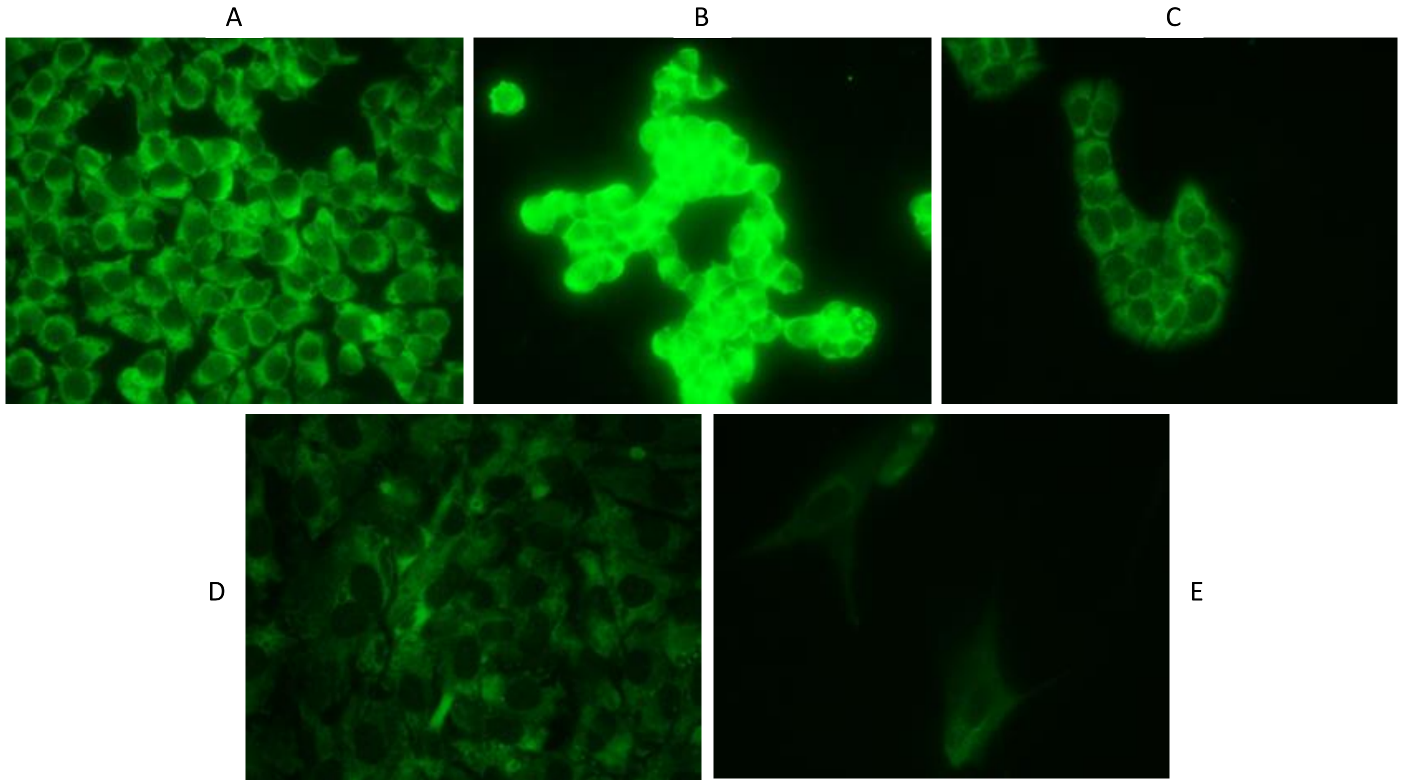

Figure 2: Immunofluorescence at 40x magnification following treatment with CBR2 antibody in A: HeLa cells, B: 293T cells, C: HaCaT cells, D: CV1 cells, and E: HDF cells. All images captured with identical expose time.

To quantitatively asses CBR2 expression, a Western Blot analysis was performed as depicted in Figure 3. The CBR2 band presented at approximately the 50kD protein marker. While all cells appeared to express some levels of CBR2, HeLa cells showed the highest expression with both 293T and HaCAT cells showing moderate expression. CV1 cells showed minimal expression with HDF cells showing slightly less expression than the CV1 cells.

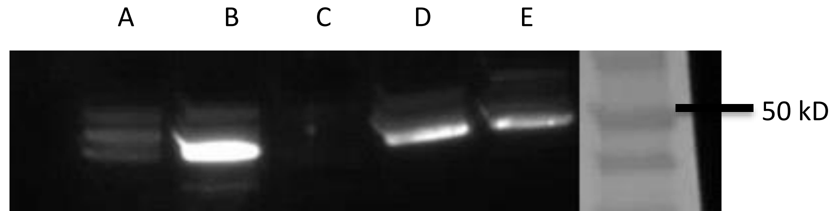

Figure 3: Western Blot data following treatment with CBR2 antibody in A: CV1 cells, B: HeLa cells, C: HDF cells, D: 293T cells, and E: HaCaT cells.

With very little expression of the CBR2 receptor in the HDF cells, it was decided that a Luciferase assay would be performed on both HeLa cells and HDF cells to elucidate whether or not the Echinacea was stimulating cell growth through the CBR2 receptor. If the proliferative properties of Echinacea were due to interactions with CBR2, then the Luciferase data for Cyclin A, Cyclin E, and p53 responsive element activation should display disparities in activation levels upon various treatments of Echinacea for the two cell lines. p53 responsive element driving luciferase acted as the negative control for HeLa cells as they are p53 null.

With a previous increase in cell proliferation upon treatment with Echinacea, it was expected that the HeLa cells would show increased Cyclin A and Cyclin E activation upon increased treatment with Echinacea. This is due to Cyclin E being responsible for allowing the cell to progress into S phase and Cyclin A allowing the cell to progress through G2 phase and into Mitosis. p53 responsive element, however, should act as a negative control in the HeLa cells and display no change in level of activation due to the ubiquitination of p53 that is caused from the Human Papilloma Virus with viral protein present in the HeLa cells. It was hypothesized that if the increase in cell growth following Echinacea treatment was due interactions with the CBR2 receptor, then there should be no changes in Cyclin A, Cyclin E and

p53 responsive element activation in the HDF cells upon varying treatments of Echinacea due to the lessened expression of CBR2 in the HDF cell line.

The results of 4 experiments in HeLa cells conducted in triplicate are seen in Figure 4. As seen in Figure 4A, HeLa cells showed a 1.30 fold increase, a 1.44 fold increase, and a 1.50 fold increase in cyclin A activity at 25, 50, and 100µg/ml respectively with both 50µg/ml and 100µg/ml treatments showing significance ($p<0.05$). As seen in Figure 4B, HeLa cells showed a 1.16 fold increase, a 1.38 fold increase, and a 1.28 fold increase in cyclin E activity at 25, 50, and 100µg/ml respectively though no treatments differed significantly from the control ($p<0.05$). As seen in Figure 4C, HeLa cells showed a significant increase in p53 responsive element activity of 1.16 fold only at 25µg/ml ($p<0.05$).

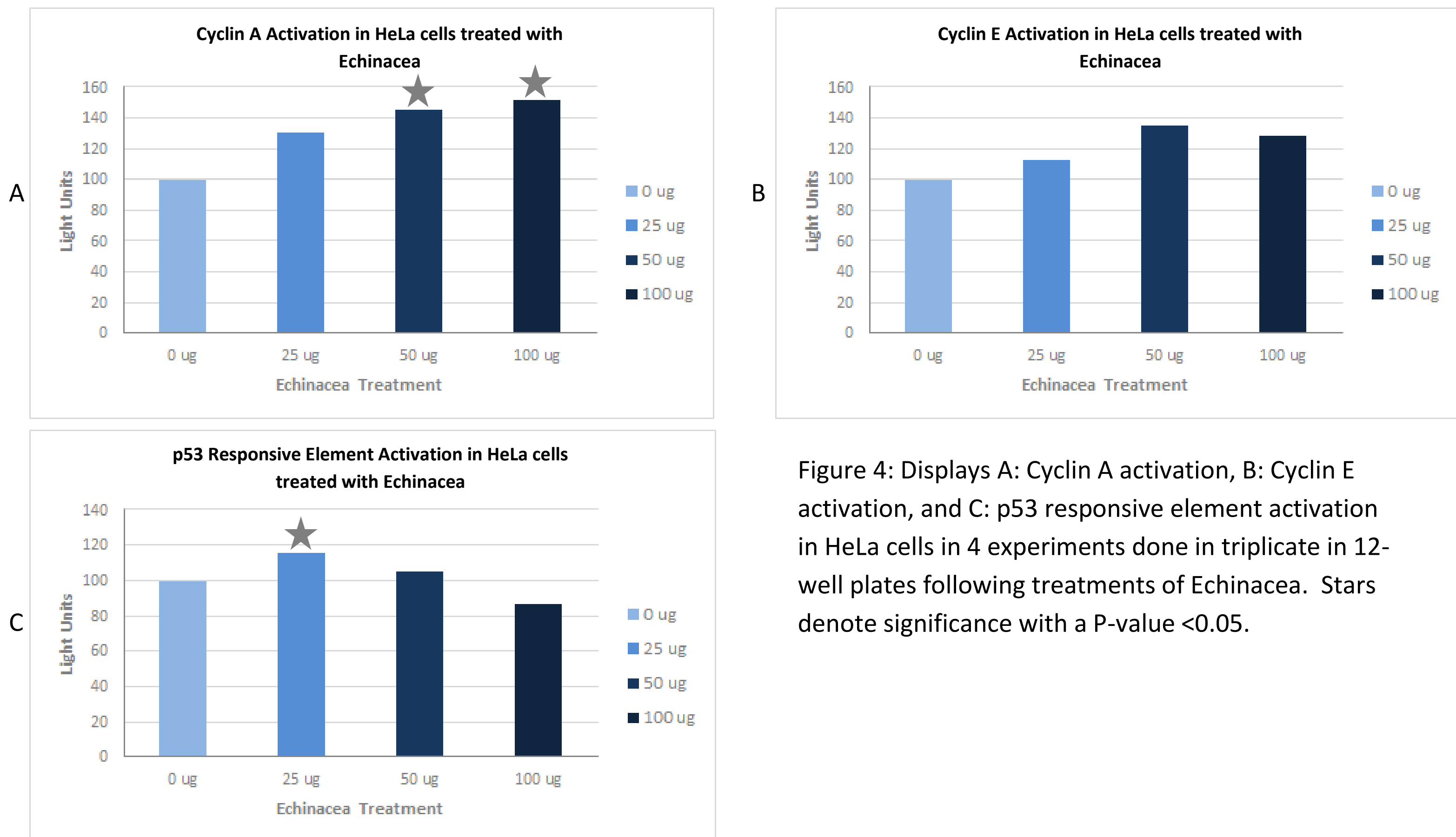

Figure 4: Displays A: Cyclin A activation, B: Cyclin E activation, and C: p53 responsive element activation in HeLa cells in 4 experiments done in triplicate in 12-well plates following treatments of Echinacea. Stars denote significance with a P-value <0.05.

The results of 4 experiments with HDF cells conducted in triplicate are seen in Figure 5. As seen in Figure 5A, HDF cells showed a 1.49 fold increase, a 1.24 fold increase, and a 1.50 fold increase in cyclin A activity at 25, 50, and 100µg/ml respectively with no treatments differing significantly from the control ($p<0.05$). As seen in Figure 5B, HDF cells showed a 2.30 fold increase, a 4.44 fold increase, and a 6.57 fold increase in cyclin E at 25, 50, and 100µg/ml respectively though no treatments differed significantly from the control ($p<0.05$). As seen in Figure 5C HDF cells showed a 1.72 fold increase, a 2.46 fold increase, and a 2.41 fold increase in p53 responsive element activity at 25, 50, and 100µg/ml respectively with only the 50µg/ml treatment differing significantly from the control ($p<0.05$).

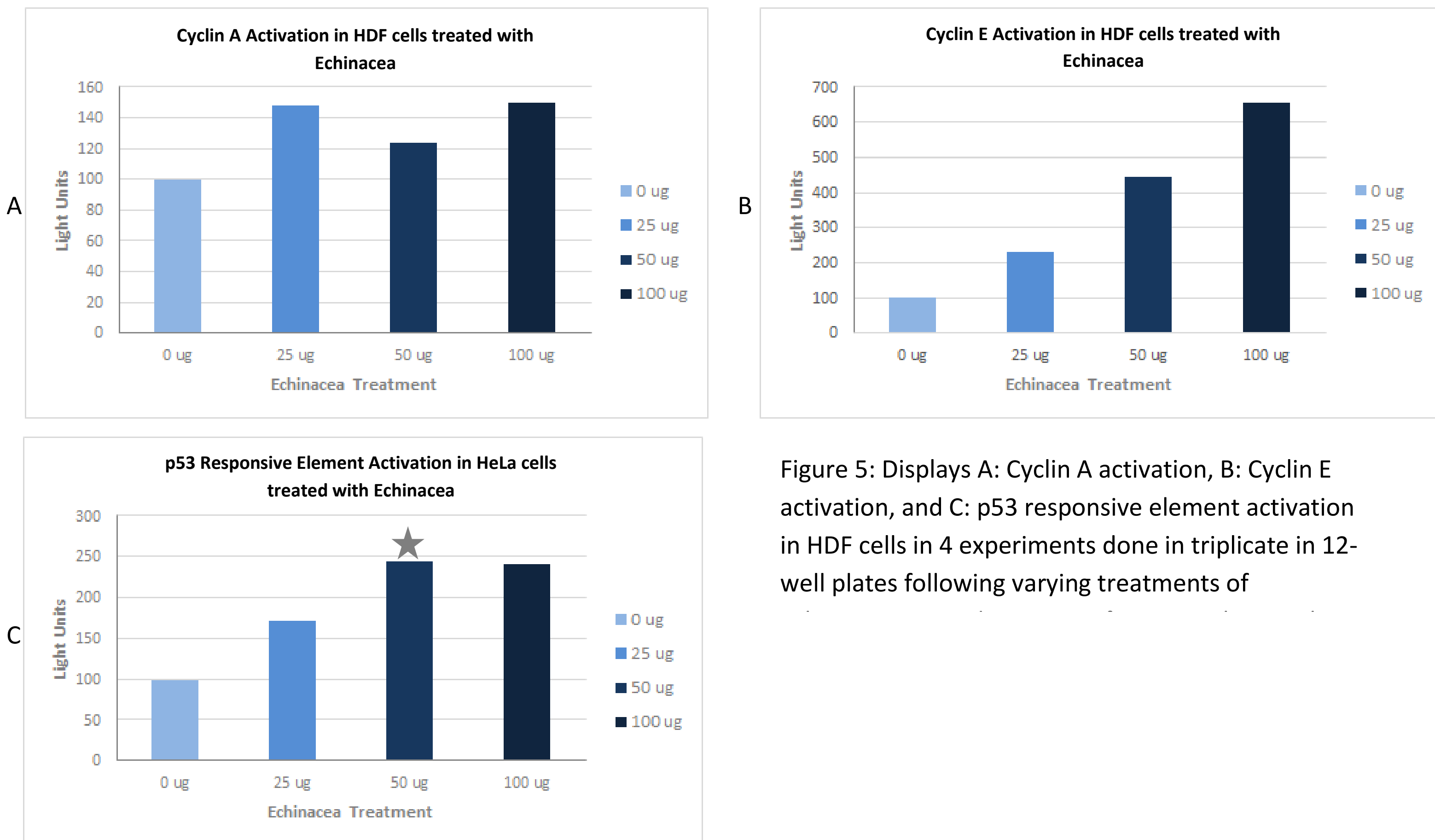

Figure 5: Displays A: Cyclin A activation, B: Cyclin E activation, and C: p53 responsive element activation in HDF cells in 4 experiments done in triplicate in 12-well plates following varying treatments of

Discussion

This study investigated the effects of Echinacea on tumor cell growth, cyclin activity, and determined CBR2 expression in five different cell lines: HeLa, 293T, HaCAT, HDF, and CV1. With Echinacea complementation with chemotherapy being commonplace today as treatment for cancer, it is alarming that this study and others found Echinacea to be increasing proliferation in certain tumor cell lines. As seen in Figure 1, upon treating HeLa cells with Echinacea, our results indicated a positive correlation between increased Echinacea treatment and increased cell proliferation. Though tumor cell growth upon treatment with this suggested chemotherapeutic compliment is alarming, our findings were consistent with previous research (Cichello et al 2015).

As seen in Figure 2, immunofluorescence utilizing CBR2 antibody treatment on HeLa, 293T, HaCAT, CV1, and HDF cells revealed CBR2 expression in all cell lines with the exception of HDF cells which displayed possible expression. A Western Blot assay, seen in Figure 3, correlated strongly with the immunofluorescence and confirmed CBR2 expression in all cell types. These results were somewhat surprising. CBR2 expression in 293T cells was not surprising as they are embryonic stem cells that have yet to differentiate. Expression of CBR2 in the HaCaT cells was expected as they are keratinocytes and CBR2 is known to be expressed by keratinocytes. Expression of CBR2 in HeLa cells was unexpected but not surprising as previous research has displayed CBR2 expression in certain tumor cell lines. Expression of CBR2 in CV1 and HDF cells was surprising as these two cell lines are not immune cells, stem cells, or tumorigenic thus that they should not be expressing the CBR2 receptor.

Our Luciferase data suggests a positive correlation between cellular proliferation and cyclin activity after treatment with Echinacea as seen in Figures 4 and 5. Romero-Sandoval et al

(2009) showed that CBR2 activation induced the MAPK pathway. Tying the two together allows for the postulated pathway, which can be seen in Figure 6. It is proposed that a constituent molecule(s) of Echinacea could activate the CBR2 receptor. The βγ subunits then activate the MAPK pathway. The MAPK pathway is then responsible for inducing cyclin E activity leading to the activation of cyclin A and thus promoting cell growth.

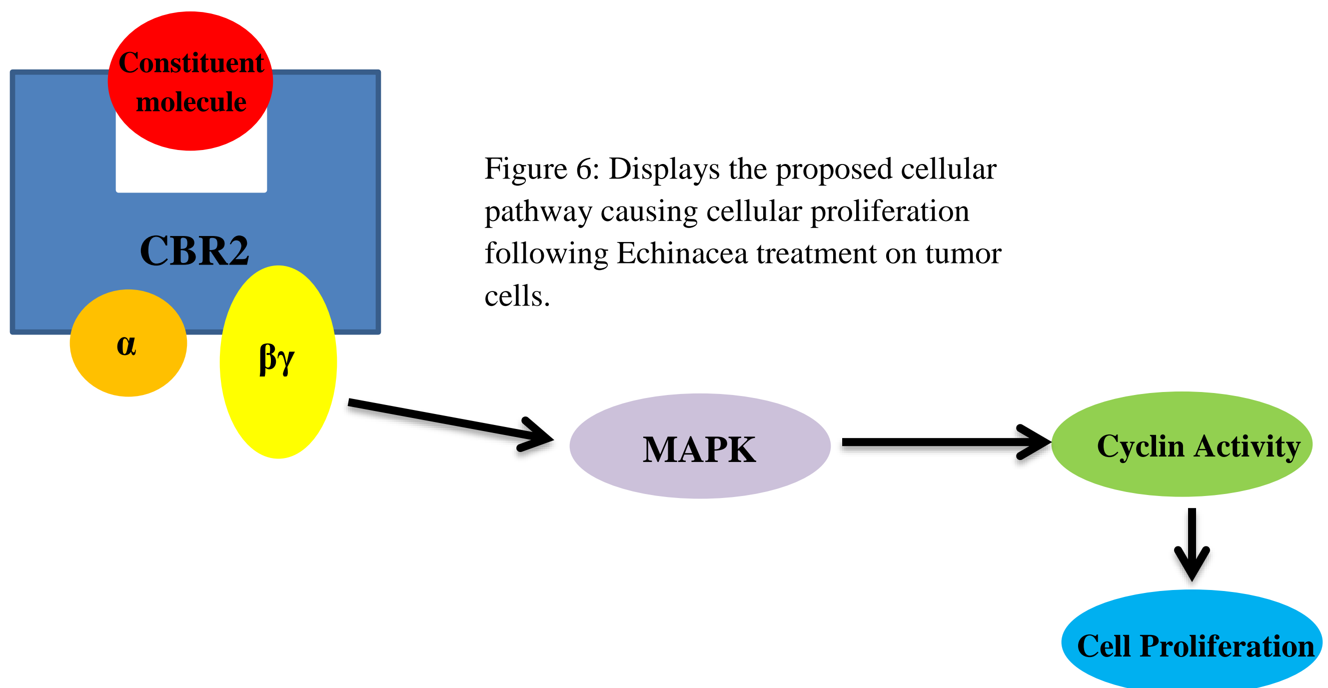

Figure 6: Displays the proposed cellular pathway causing cellular proliferation following Echinacea treatment on tumor cells.

To further elucidate the role that the CBR2 receptor plays in Echinacea signaling Western blotting can be utilized to assess for Akt phosphorylation and cyclic AMP can be monitored for increases or decreases to determine the downstream effects Echinacea has on CBR2 signaling. Additionally, cell counts must be taken to determine the effect of Echinacea treatment on HDF cell growth. With wide variability in the Luciferase data, repeat experiments must be performed to obtain more concrete evidence of promoter activation in the HeLa and HDF cell lines.

The increase in tumor cell growth following Echinacea treatment can finally be analyzed by performing Echinacea fractionation. By separating the Echinacea into its constituent molecules, the constituent molecules responsible for the increased proliferation will be able to be exposed. To do this the constituent molecules could be directly applied to the cells and a change in proliferation could be measured.

Conclusions

With the widespread use of Echinacea supplementation as a complement to chemotherapeutic treatment in cancer patients, it should be of great concern that Echinacea treatment on tumor cells is causing increased proliferation. Our data supports previous findings of increased cell proliferation in HeLa cells upon treatment with Echinacea further revealing the need for more research on the effects of Echinacea. Our lab was able to definitively demonstrate CBR2 expression in several commonly used cell lines and is currently working to determine whether there is expression in the HDF cell line. While preliminary Luciferase data was not concrete, it does seem to suggest that the increased proliferation seen upon Echinacea treatment is, at least, partially caused by Echinacea interacting with CBR2.

Works Cited

Approved Drug Products with Therapeutic Equivalence Evaluations (Orange Book). (2005). Retrieved from:http://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm

Block, Keith I., and Mark N. Mead. "Immune System Effects of Echinacea, Ginseng, and Astragalus: A Review." *Integr Cancer Ther Integrative Cancer Therapies* 2.3 (2003): 247-67. Web

Cichello, Simon Angelo, Qian Yao, and Xiao Qiong He. "Proliferative Activity of a Blend of Echinacea Angustifolia and Echinacea Purpurea Root Extracts in Human Vein Epithelial, HeLa, and QBC-939 Cell Lines, but Not in Beas-2b Cell Lines." *Journal of Traditional and Complementary Medicine* 6.2 (2016): 193-97. Web.

Echinacea. (2012) Retrieved from: https://nccih.nih.gov/health/echinacea/ataglance.htm

Gertsch, Juerg, Roland Schoop, Urs Kuenzle, and Andy Suter. "Echinacea Alkylamides Modulate TNF-α Gene Expression via Cannabinoid Receptor CB2 and Multiple Signal Transduction Pathways." *FEBS Letters* 577.3 (2004): 563-69. Web.

Huntimer, Eric D., Fathi T. Halaweish, and Christopher C. L. Chase. "Proliferative Activity OfEchinacea Angustifolia Root Extracts on Cancer Cells: Interference with Doxorubicin Cytotoxicity." *C&B Chemistry & Biodiversity* 3.6 (2006): 695-703. Web.

Lersch, C., M. Zeuner, K. Siebenrock, R. Hart, F. Wagner, U. Fink, H. Dancygier, and M. Classen. "Stimulation of the Immune Response in Outpatients with Hepatocellular Carcinomas by Low Doses of Cyclophosphamide (LDCY), Echinacea Purpurea Extracts (Echinacin) and Thymostimulin." *Arch Geschwulstforsch* 60.5 (1990): 379-83. Web.

Marlies, K., Bruce, B,. David, K., Rudolf, B., Karin, A., Klaus, L., (2014) Echinacea for preventing and treating the common cold. Cochrane Database of Systematic Reviews. John Wiley & Sons, Ltd

Miller, Lucinda G. "Herbal Medicinals." *Arch Intern Med Archives of Internal Medicine* 158.20 (1998): 2200. Web.

Mistríková, Ingrid, and Štefánia Vaverková. "Echinacea – Chemical Composition, Immunostimulatory Activities and Uses."*Thaiszia Journal of Botany* (2006): 11-26. Web.

Monory, K., M. Polack, A. Remus, B. Lutz, and M. Korte. "Cannabinoid CB1 Receptor Calibrates Excitatory Synaptic Balance in the Mouse Hippocampus." *Journal of Neuroscience* 35.9 (2015): 3842-850. Web.

Perez-Gomez, E., C. Andradas, S. Blasco-Benito, M. M. Caffarel, E. Garcia-Taboada, M. Villa-Morales, E. Moreno, S. Hamann, E. Martin-Villar, J. M. Flores, A. Wenners, I. Alkatout, W. Klapper, C. Rocken, P. Bronsert, E. Stickeler, A. Staebler, M. Bauer, N. Arnold, J.

Soriano, M. Perez-Martinez, D. Megias, G. Moreno-Bueno, S. Ortega-Gutierrez, M. Artola, H. Vazquez-Villa, M. Quintanilla, J. Fernandez-Piqueras, E. I. Canela, P. J. Mccormick, M. Guzman, and C. Sanchez. "Role of Cannabinoid Receptor CB2 in HER2 Pro-oncogenic Signaling in Breast Cancer." *JNCI Journal of the National Cancer Institute* 107.6 (2015): n. pag. Web.

Preet, A., Z. Qamri, M. W. Nasser, A. Prasad, K. Shilo, X. Zou, J. E. Groopman, and R. K. Ganju. "Cannabinoid Receptors, CB1 and CB2, as Novel Targets for Inhibition of Non-Small Cell Lung Cancer Growth and Metastasis." *Cancer Prevention Research* 4.1 (2010): 65-75. Web.

Raduneretal. Alkylamides from Echinacea are a new class of Cannabinomimetics. *The Journal of Biological Chemistry, 281* (20), 14192-14206.

Romero-Sandoval, Alfonso, Nancy Nutile-Mcmenemy, and Joyce A. Deleo. "Spinal Microglial and Perivascular Cell Cannabinoid Receptor Type 2 Activation Reduces Behavioral Hypersensitivity without Tolerance after Peripheral Nerve Injury." *Anesthesiology* 108.4 (2008): 722-34. Web.

Romero-Sandoval, Edgar, Ryan Horvath, Russell P. Landry, and Joyce A. Deleo. "Cannabinoid Receptor Type 2 Activation Induces a Microglial Anti-inflammatory Phenotype and Reduces Migration via MKP Induction and ERK Dephosphorylation." *Molecular Pain Mol Pain* 5.1 (2009): 25. Web.

Samson, M.-T., A. Small-Howard, L. M. N. Shimoda, M. Koblan-Huberson, A. J. Stokes, and H. Turner. "Differential Roles of CB1 and CB2 Cannabinoid Receptors in Mast Cells." *The Journal of Immunology* 170.10 (2003): 4953-962. Web.

Shah, Sachin A., Stephen Sander, C. Michael White, Mike Rinaldi, and Craig I. Coleman. "Evaluation of Echinacea for the Prevention and Treatment of the Common Cold: A Meta-analysis." *The Lancet Infectious Diseases* 7.7 (2007): 473-80. Web.

Sim-Selley, Laura J. "Regulation of Cannabinoid CB1 Receptors in the Central Nervous System by Chronic Cannabinoids." *Crit Rev Neurobiol Critical Reviews in Neurobiology* 15.2 (2003): 91-119. Web.

Wang C-Y, Chiao M-T, Yen P-J, Huang W-C, Hou C-C, Chien S-C, et al. “Modulatory effects of Echinacea purpurea extracts on human dendritic cells: a cell- and gene-based study.” *Genomics*. 2006; 88:801–8.