Research Article

"Full spectrum cannabidiol oil reduces breast cancer cell viability and expression of epidermal growth factor receptor."

Anastasia H. Smith, Mary Claire Cooperrider, Ashton R. Hogue, Nathaniel B. Hunter, Diego De La Torre, Madeline P. McTigue, and William P. Ranahan II. Oral Roberts University Tulsa, OK 74171. Correspondence to wranahan@oru.edu. Running header: CBD oil and breast cancer
Key Words: CBD, EGFR, hemp,

Abstract

Full spectrum CBD oil is widely available and touted as a safe and effective way to lower anxiety, promote restful sleep, and reduce inflammation. Given the anecdotal evidence that full spectrum CBD oil can also be used as an adjuvant to chemotherapy, an over-the-counter CBD oil product was tested to determine if it possessed cell cytotoxic properties. A "triple negative" breast cancer cell line was chosen as it represents a breast cancer subset which lacks targeted therapies and correlates with poor clinical outcomes. Cancer cells exposed to CBD oil had reduced cell viability, broad changes in gene expression, and reduced expression of a key growth factor receptor compared to control cells. Together, these data suggest that CBD oil is effective at reducing breast cancer cell viability *in vitro*. Future studies will focus on determining the range of cancer cell types similarly affected and on confirming mechanistic details.

Introduction

Cancer is the second leading cause of death worldwide, second only to cardiovascular disease (Jemal et al. 2011). Although significant time and resources have been invested into cancer research, our standard operating procedures remain problematic for healthy tissues (Pérez-Herrero and Fernández-Medarde 2015). While several subsets of cancer have targeted therapies, the majority of cancer cases are treated with chemotherapies which are toxic to non-cancerous cells. Drugs which can selectively target and destroy cancer cells remain the ultimate goal of cancer researchers worldwide (Walcher et al. 2020; Guerra-Martín et al. 2021). In general, naturally derived substances have fewer off target effects when properly administered. First world countries like Japan and China routinely use herbal formulations as adjuvant therapies for cancer treatment (Liu et al. 2011). One such naturally occurring class of compounds, which has been gaining attention since the 1960s, is the family of biologically active lipids derived from the plant *Cannabis sativa*. These compounds are collectively referred to as cannabinoids. Following the 2018 Agriculture Improvement Act or "Farm Bill", hemp i.e. *Cannabis sativa* L., and hemp derived products with <0.3% tetrahydrocannabinol (THC), are considered commodities (Alharbi 2020). Since that time, over-the-counter dietary supplements containing full spectrum cannabidiol oil (fsCBD) have become increasingly popular. While CBD has been shown to reduce inflammation and promote restful sleep, its effects on cancer cells via the CB1 and CB2 receptors is an area of

active investigation (VanDolah et al. 2019) (Hermanson and Marnett 2011). Students enrolled in the Special Topics: Cancer Biology class were tasked with finding an over-the-counter dietary supplement which may possess cell cytotoxic properties on cancer cells. Given the lack of published data and the wide use and availability of fsCBD products, an oil-based extract of fsCBD was selected. The mammary epithelial cell line MDAMB-468 was selected as a model of "triple negative" breast cancer (TNBC). Triple negative breast cancers lack the targetable receptors common in many forms of breast cancer i.e. EGFR2, progesterone, and the estrogen receptor. TNBC has very poor clinical outcomes (CDC Breast Cancer 2022 Mar 9). Furthermore, TNBC cells have been reported to be sensitive to synthetic cannabinoid derivatives (Greish et al. 2018). The authors hypothesized that fsCBD oil would reduce breast cancer cell viability and elicit broad changes in gene expression.

Materials and Methods

Full-spectrum CBD oil was a kind gift from AgraPharm LLC.

Mammalian cell culture. MDAMB468 cells were purchased from ATCC (HTB-132) and cultured in Leibovitz-15 supplemented with 10% FBS and 1% antibiotic/antimycotic at 37°C. Cells used in this study were passaged less than 40 times following purchase from ATCC.

Cell viability assays. Cell viability was assessed with Abcam Cell Cytotoxicity assay per manufacture's instructions. Briefly, twenty thousand cells were seeded into wells of a 96 well plate. After 24 hours cells were exposed to a 2% solution (V/V) of CDB oil or control vehicle. 24 hours after treatment Abcam reagent was added. Cell viability was measured on a 96 well spectrophotometer (BioRad) at wavelengths of 570 and 605nm respectively. Cell viability was calculated as a ratio of 570/605nm absorptivity.

RNA extraction, purification, and cDNA generation. 24 hours following treatment, cell media was aspirated. Cells were washed with PBS and RNA was extracted via the phenol chloroform method. RNA purity and concentration was determined with a NanoDrop spectrophotometer. cDNA was generated from 1ug total RNA via iScript cDNA Synthesis Kit (BioRad).

Quantitative PCR. PrimePCR Breast Neoplasms Tiers 1-3 H96 Arrays (BioRad) were run per manufacture's instructions. Briefly, master mix containing SsoAdvanced Universal SYBR Green Supermix (BioRad) was added to each well containing pre-validated primer pairs, followed by a volume (2ul) of cDNA representative of 100ng total RNA. 96-well plates were thermal cycled once for 2 minutes at 95°C, then 40 times at 95°C for 5 seconds to denature followed by a combined annealing and extension step at 60°C for 30 seconds. qPCR parameters concluded with a melt curve which began at 65°C and increased to 95°C every 5 seconds. Primer sequences for PrimePCR Neoplasms Tiers 1-3 are available at BioRad.com. Primer sequences for select reproduced targets are as follows: BCL2 Fwd gagctggtggttgactttctc Rev tccatctccgattcagtccct MMP9 Fwd tgtaccgctatggttacactcg Rev ggcagggaccgttgcttct PCNA Fwd cagcggtaggtgtcgaagc Rev cagcggtaggtgtcgaagc IL6 Fwd ggcactggcagaaaacaacc Rev gcaagtctcctcattgaatcc EGFR Fwd

acctgcgtgaagaagtgtc Rev cgtcttcctccatctcatagc HMOX1 Fwd gcccagatcctctcacttaatc Rev cccagcttcctatcctatccta

Immunobloting. Cells were treated with a 2% (V/V) control or CBD oil solution and allowed to incubate overnight at 37°C. Media was aspirated and cells were washed with PBS. Clarified cell lysates were generated with RIPA lysis buffer supplemented with a protease inhibitor cocktail (Sigma). Following lysis, cells were centrifuged at 15,000Xg for 10 minutes at 4°C. Protein concentrations were determined with a bicinchoninic acid assay (BCA). Samples were loaded in duplicate on 7.5% Stain-Free FastCast gels (BioRad) and run at 150V. Gels were transferred to nitrocellulose membranes (Sigma) with the Trans-Blot Turbo Transfer System (BioRad). Membranes were probed with antibodies, at a 1 to 1000 dilution, directed against β-actin (Cell Signaling) and EGFR (Cell Signaling) respectively. Visualization of proteins was accomplished with chemiluminescence on a ChemiDoc Imaging System (BioRad).

Results

MDAMB-468 cells were treated with various concentrations of fsCBD oil or control vehicle, i.e. olive oil. Cells exposed to 2% (v/v) or higher fsCBD oil showed dramatically reduced viability after overnight incubations compared to control. Cell viability assays were completed with 5 technical replicates per condition. Each technical replicate contained 20,000 cells. Each viability assay was repeated at least 3 times to ensure accuracy and reproducibility. Average percent cell viabilities are shown relative to control populations (Fig 1). TNBC cells have upregulated expression of the cannabinoid receptors CB1 and CB2 (Khunluck et al. 2022). The susceptibility of TNBC cells to these agonists has only been recently appreciated. The endocannabinoid system itself has only recently been discovered and the resultant effects of CB1 and CB2 activation in cancer cells is still being elucidated (Laezza et al. 2020). In order to directly measure the changes in gene expression following fsCBD treatment, quantitative polymerase chain reaction (qPCR) was employed. Complimentary DNA (cDNA) was generated from populations of MDAMB-468 cells treated with a 2% (V/V) solution of fsCBD oil or control vehicle. Total RNA was extracted, purified, and converted into cDNA via reverse transcriptase. Template cDNA was added to Prime PCR arrays. Prime PCR arrays provide a convenient and validated approach to measuring changes in gene expression. 96-well PCR plates were purchased pre-loaded with validated primers. In this case, the top 3 tiers of cancer genes driving breast cancer malignancy were assayed. Given the large number of targets, data are shown as heat map images (Fig 2 left column) and fold changes in gene expression relative to control cells (Fig 2 right columns). Six targets, which play significant roles in mammary tumorigenesis, were chosen for further validation. Subsequent select qPCR assays revealed similar results (data not shown). One target that consistently showed mRNA upregulation in MDAMB-468 cells treated with fsCBD oil was EGFR. The observed increase in EGFR mRNA transcripts repeated 3 times and was unexpected. A previous study, looking at CB2 in breast cancer, has provided evidence that exposure of TNBC cells to a synthetic version of a CB2 specific agonist resulted in decreased EGFR expression (Elbaz et al. 2016). Given that increases in mRNA levels are not necessarily correlated with increases in protein expression, treated and control cells

were assayed for EGFR protein levels (Fig 4). A dramatic decrease in total EGFR protein was consistently observed following fsCBD treatment.

Discussion

Efficacy of fsCBD oil at reducing MDMBA-468 cell viability was consistent and surprising. Given that fsCBD oil is routinely used topically and orally it is surprising that this dietary supplement is thus effective. Several genes consistently upregulated expression following treatment with fsCBD oil compared to treatment (Fig 2). Of particular interest to the authors were heme oxygenase-1 (HMOX1), interleukin 6 (IL6), and epidermal growth factor receptor (EGFR). These targets, in particular, had dramatic or unexpected increases in expression (Fig 3). HMOX1 is a heme oxygenase which is essential in heme catabolism. Interestingly, HMOX1 expression correlates strongly with poor clinical outcomes (Hassannia et al. 2019). IL6 is involved in immune system functions and mediates inflammation. Its role in cancer has been linked to its mediation of the inflammatory response, particularly via signal transducer and activator of transcription 3 (STAT3) signaling (Hirano 2021). It is unclear whether the increase in IL6 is due to surviving cells being surrounded by necrotic cells, and thus is indirectly increasing, or the increase is due to fsCBD oil directly increasing IL6 expression. Increases in IL6 following CB1/CB2 activation have been reported, although the mechanisms remain unclear (Klein et al. 2000). The EGFR is of particular interest as TNBC lacks three of the major growth receptors. Cell viability data clearly showed an overall decrease when cells were treated with fsCBD oil (Fig 1). Given that EGFR is a key mediator of the mitogen activated protein kinase (MAPK) pathway, which promotes cell replication, its increase in mRNA expression was unexpected. The observed increase in mRNA transcripts of EGFR repeated 3 times. Immuno blot analyses, however, showed a dramatic decrease in total EGFR protein levels (Fig 4). These data are consistent with a report of decreased EGFR protein levels in TNBC cells treated with a synthetic cannabinoid (Elbaz et al. 2016). It is also noteworthy that treatment of TNBC cells with fsCBD oil resulted in an approximately 20-fold increase in small ubiquitin like modifier 1 (SUMO1). SUMO1 is involved in a wide range of cellular processes such as transcriptional regulation, protein stability, and apoptosis (Han et al. 2018; Chang and Yeh 2020). It is possible that the increase in SUMO1 expression is directly impacting the protein stability of the EGFR.

Several transcriptional targets decreased in expression following treatment with fsCBD oil. Among those targets were proliferating cell nuclear antigen (PCNA), B-cell lymphoma 2 (Bcl2), and matrix metallopeptidase 9 (MMP9). PCNA is a DNA polymerase cofactor which facilitates leading strand synthesis during DNA replication. PCNA expression directly correlates with cell replication (González-Magaña and Blanco 2020). The observed decrease in PCNA mRNA levels is consistent with the cell viability data suggesting treatment of MDAMB-468 cells with fsCBD oil reduces cell viability compared to control cells. BCL2 is a regulator of apoptosis whose expression is associated with several types of cancer (Singh et al. 2019). BCL2 is antiapoptotic, therefore the decrease in its expression is consistent with a decrease in cancer cell viability. MMP9 is a zinc metalloproteinase family member which functions to degrade the extracellular matrix (ECM). Degradation of the ECM is a required step facilitating migration during tumorigenesis (Huang 2018). A decrease in

MMP9 expression suggests that MDAMB-468 cells have a decreased invasive capacity following treatment with fsCBD oil.

Breast cancer is estimated to effect 1 in 3 women in the United States alone (DeSantis et al. 2019). TNBC, which make up approximately 10-15% of breast cancer cases, has a poor clinical outcome percentage and is particularly a burden for minority women (Scott et al. 2019). Given the lack of available treatments without significant side effects, naturally occurring alternatives should be explored. The authors conclude that while fsCBD oil is well tolerated in both oral and topical applications, its effect on cancer cells warrants further investigation.

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References

Alharbi YN. 2020. Current legal status of medical marijuana and cannabidiol in the United States. Epilepsy Behav. 112:107452. doi:10.1016/j.yebeh.2020.107452.

CDCBreastCancer. 2022 Mar 9. Triple-Negative Breast Cancer. Centers for Disease Control and Prevention. [accessed 2022 Jul 18]. https://www.cdc.gov/cancer/breast/triple-negative.htm.

Chang H-M, Yeh ETH. 2020. SUMO: From Bench to Bedside. Physiol Rev. 100(4):1599–1619. doi:10.1152/physrev.00025.2019.

DeSantis CE, Ma J, Gaudet MM, Newman LA, Miller KD, Goding Sauer A, Jemal A, Siegel RL. 2019. Breast cancer statistics, 2019. CA: A Cancer Journal for Clinicians. 69(6):438–451. doi:10.3322/caac.21583.

Elbaz M, Ahirwar D, Ravi J, Nasser MW, Ganju RK. 2016. Novel role of cannabinoid receptor 2 in inhibiting EGF/EGFR and IGF-I/IGF-IR pathways in breast cancer. Oncotarget. 8(18):29668–29678. doi:10.18632/oncotarget.9408.

González-Magaña A, Blanco FJ. 2020. Human PCNA Structure, Function and Interactions. Biomolecules. 10(4):E570. doi:10.3390/biom10040570.

Greish K, Mathur A, Al Zahrani R, Elkaissi S, Al Jishi M, Nazzal O, Taha S, Pittalà V, Taurin S. 2018. Synthetic cannabinoids nano-micelles for the management of triple negative breast cancer. Journal of Controlled Release. 291:184–195. doi:10.1016/j.jconrel.2018.10.030.

Guerra-Martín MD, Tejedor-Bueno MS, Correa-Casado M. 2021. Effectiveness of Complementary Therapies in Cancer Patients: A Systematic Review. Int J Environ Res Public Health. 18(3):1017. doi:10.3390/ijerph18031017.

Han Z-J, Feng Y-H, Gu B-H, Li Y-M, Chen H. 2018. The post-translational modification, SUMOylation, and cancer (Review). Int J Oncol. 52(4):1081–1094. doi:10.3892/ijo.2018.4280.

Hassannia B, Vandenabeele P, Vanden Berghe T. 2019. Targeting Ferroptosis to Iron Out Cancer. Cancer Cell. 35(6):830–849. doi:10.1016/j.ccell.2019.04.002.

Hermanson DJ, Marnett LJ. 2011. Cannabinoids, endocannabinoids, and cancer. Cancer Metastasis Rev. 30(3–4):599–612. doi:10.1007/s10555-011-9318-8.

Hirano T. 2021. IL-6 in inflammation, autoimmunity and cancer. Int Immunol. 33(3):127–148. doi:10.1093/intimm/dxaa078.

Huang H. 2018. Matrix Metalloproteinase-9 (MMP-9) as a Cancer Biomarker and MMP-9 Biosensors: Recent Advances. Sensors (Basel). 18(10):E3249. doi:10.3390/s18103249.

Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. 2011. Global cancer statistics. CA Cancer J Clin. 61(2):69–90. doi:10.3322/caac.20107.

Khunluck T, Lertsuwan K, Chutoe C, Sooksawanwit S, Inson I, Teerapornpuntakit J, Tohtong R, Charoenphandhu N. 2022. Activation of cannabinoid receptors in breast cancer cells improves osteoblast viability in cancer-bone interaction model while reducing breast cancer cell survival and migration. Sci Rep. 12(1):7398. doi:10.1038/s41598-022-11116-9.

Klein TW, Lane B, Newton CA, Friedman H. 2000. The cannabinoid system and cytokine network. Proc Soc Exp Biol Med. 225(1):1–8. doi:10.1046/j.1525-1373.2000.22501.x.

Laezza C, Pagano C, Navarra G, Pastorino O, Proto MC, Fiore D, Piscopo C, Gazzerro P, Bifulco M. 2020. The Endocannabinoid System: A Target for Cancer Treatment. Int J Mol Sci. 21(3):747. doi:10.3390/ijms21030747.

Liu Jianping, Li Xun, Liu Jingyuan, Ma L, Li Xinxue, Fønnebø V. 2011. Traditional Chinese medicine in cancer care: a review of case reports published in Chinese literature. Forsch Komplementmed. 18(5):257–263. doi:10.1159/000333065.

Pérez-Herrero E, Fernández-Medarde A. 2015. Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. Eur J Pharm Biopharm. 93:52–79. doi:10.1016/j.ejpb.2015.03.018.

Scott LC, Mobley LR, Kuo T-M, Il'yasova D. 2019. Update on Triple Negative Breast Cancer Disparities for the United States – A Population Based Study from the United States Cancer Statistics database, 2010-2014. Cancer. 125(19):3412–3417. doi:10.1002/cncr.32207.

Singh R, Letai A, Sarosiek K. 2019. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. Nat Rev Mol Cell Biol. 20(3):175–193. doi:10.1038/s41580-018-0089-8.

VanDolah HJ, Bauer BA, Mauck KF. 2019. Clinicians' Guide to Cannabidiol and Hemp Oils. Mayo Clin Proc. 94(9):1840–1851. doi:10.1016/j.mayocp.2019.01.003.

Walcher L, Kistenmacher A-K, Suo H, Kitte R, Dluczek S, Strauß A, Blaudszun A-R, Yevsa T, Fricke S, Kossatz-Boehlert U. 2020. Cancer Stem Cells-Origins and Biomarkers: Perspectives for Targeted Personalized Therapies. Front Immunol. 11:1280. doi:10.3389/fimmu.2020.01280.

Figure Legends

Figure 1. Full spectrum CBD oil reduces "triple negative" mammary epithelia cancer cell viability in-vitro. MDAMB-468 cells were exposed to 4,8, or 12% solutions (v/v) of full-spectrum CBD oil or control vehicle, respectively, for 24 hours before assessment of cell viability. Error bars indicate standard deviation of the mean. Asterisk indicate significance between control and treatment populations as determined by Student's T-test p-value of less than 0.01.

Figure 2. Full spectrum CBD oil exposure results in broad changes in gene expression. A,B,C. Prime PCR Breast Neoplasm Tiers 1,2, and 3 respectively. cDNA from MDAMB-468 cells treated with full spectrum CBD oil or vehicle control were analyzed via qPCR. Validated gene targets were assayed with BioRad Prime PCR arrays. Decreases in gene expression relative to control indicated in green. Increases in gene expression indicated in red. Black indicates no change and grey indicates no data. Fold change indicated is relative to control.

Figure 3. Select changes in gene expression following exposure to full-spectrum CBD oil. Representative Prime PCR targets from each tier shown. Changes in gene expression are relative to control targets set to 1. Select targets were confirmed via qPCR with alternative primer pairs following Prime PCR analysis.

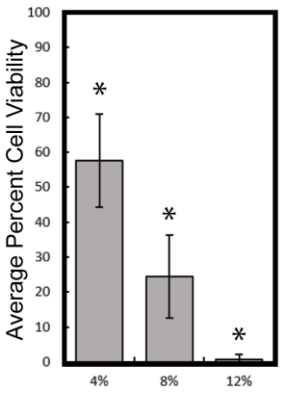
Figure 4. Full spectrum CBD oil reduces EGFR protein in-vitro. MDA MB-468 cells were treated with a 2% solution (V/V) full spectrum CBD oil or control vehicle for 24 hours before immunoblot analysis. A. 40ug of protein were analyzed per sample. Samples loaded in duplicate. B. Pixel density of three independent experiments was analyzed. Error bars are standard deviation of the mean. Asterisk indicate significance between control and treatment populations as determined by Student's T-test p-value of less than 0.01.

Author Contributions

AHS, MCC, ARH, and NBH performed the cell viability and PrimePCR assays. DDLT and MPM repeated the cell viability and qPCR assays as well as helped with the immunoblot assays. WPR directed the study and wrote the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflicts of interest.



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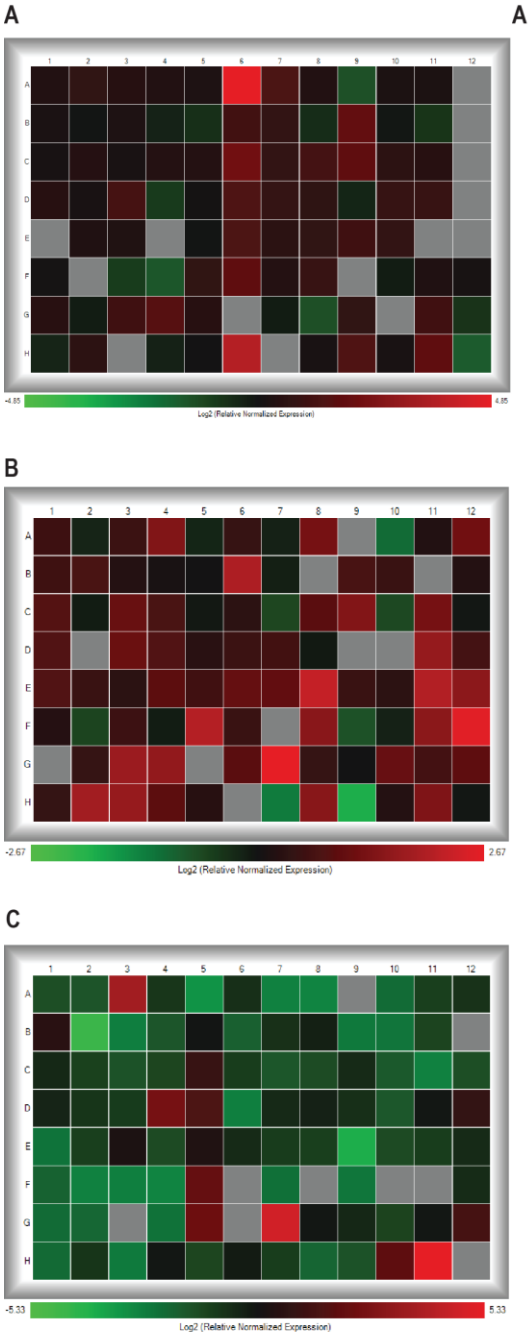

A

Target	Fold Change
HMOX1	28.75
IL6	13.44
IGF1	4.49
PLAU	3.59
VIM	3.32
PPARG	3.32
IL18	3.32
EGFR	2.98
SERPINE1	2.68
IGFBP3	2.63
IL10	2.56
IL8	2.53
CDKN1A	2.24
MYC	2.21
ICAM1	2.12
VEGFA	2.10
COL1A1	2.07
PTEN	2.05
NFKBIZ	1.81
UBC	1.80
STAT3	1.78
MAPK1	1.73
MAP2K1	1.67
JUN	1.66
TGFB1	1.64
RELA	1.63
NFKBIA	1.62
BCL2L1	1.61
GSK3B	1.59
NFKB1	1.58
CCND1	1.53
STAT1	1.52
MAPK3	1.48
BAX	1.40
APOE	1.39
GSTP1	1.38
UBB	1.37
CD44	1.35
BRCA1	1.33
CTNNB1	1.32
MDM2	1.32
EZH2	1.31
ABCB1	1.28
MMP2	1.27
CREB1	1.27
VDR	1.23
CAV1	1.23
CDKN1B	1.22
CDH1	1.19
ERBB2	1.18
TNFRSF1A	1.15
SOD2	1.15
ACTB	1.13
CDK1	1.10
NR3C1	1.10
TNF	1.10
CASP3	1.09
AKT1	1.09
RQ1	1.08
AURKA	1.05
FN1	1.03
HIF1A	1.01
BIRC5	-1.03
FOS	-1.03
SPP1	-1.06
CCL5	-1.15
TIMP1	-1.16
MKI67	-1.16
EGR1	-1.24
CTGF	-1.25
BCL2	-1.28
PROM1	-1.31
MMP9	-1.46
ESR1	-1.55
RQ2	-1.64
TYMS	-1.67
CXCL10	-1.80
CDKN2A	-1.87
NOS2	-2.56
PCNA	-2.60
CXCR4	-2.83
RT	-3.08

B

Target	Fold Change
NOTCH1	6.35
WNT5A	6.00
PLAUR	4.71
KDR	4.16
TIMP2	4.08
MAP2K2	3.89
CDK4	3.54
EDN1	3.28
ERCC1	3.13
THBS1	3.10
HRAS	3.04
TWIST1	2.91
TIMP3	2.85
PPARA	2.80
PRKCA	2.77
RASSF1	2.64
FGF2	2.60
TNC	2.59
TGFBR2	2.40
PDPK1	2.38
TNFAIP3	2.29
DAPK1	2.15
CYR61	2.10
SRC	2.09
MGMT	2.06
NFKB2	2.01
XPO5	1.93
HSPA5	1.88
GSTM5	1.88
MLH1	1.87
PIK3CA	1.87
ATF2	1.78
GPR22	1.75
ATM	1.75
BDNF	1.73
GALNT11	1.63
CCNB1	1.63
RAF1	1.61
TUBB2C	1.55
TLR2	1.55
NFE2L2	1.53
KDM1A	1.49
APP	1.47
ACTA2	1.46
E2F1	1.45
MET	1.43
CEBPB	1.43
CCR5	1.41
SFN	1.41
RFC4	1.41
MIF	1.41
MAP1LC3B	1.38
CDK2	1.36
PRKACB	1.36
CBX7	1.35
SOCS3	1.26
MCL1	1.26
DNMT1	1.25
JUNB	1.21
LGALS3	1.19
BUB1B	1.18
CYP20A1	1.16
TACC3	1.16
TOP2A	1.15
TCF7L2	1.14
FOXM1	1.05
ITGB1	1.01
S100A4	1.00
TPX2	-1.02
ZWINT	-1.03
ITPR1	-1.05
PIK3R1	-1.05
HLA-DRA	-1.08
CCNB2	-1.08
MSH2	-1.10
SPARC	-1.12
MMP7	-1.12
CCNA2	-1.14
IGF1R	-1.16
CD14	-1.52
MUC1	-1.55
SNAI1	-1.59
RRM2	-1.71
S100A9	-2.13
PDGFRA	-2.48
S100A8	-3.81

C

Target	Fold Change
SUMO1	40.34
MAD2L1	26.96
CDC42EP4	12.57
DKK1	5.69
GJA1	4.88
GDF15	4.27
SFRP1	3.66
GADD45A	2.84
UCHL1	2.45
FYN	1.91
UBE2C	1.78
ABCA1	1.46
GADD45B	1.24
CENPE	1.17
FLT1	-1.04
NQO1	-1.05
SSBP1	-1.06
SLC25A5	-1.06
FGFR1	-1.06
IRF1	-1.15
MELK	-1.24
NAMPT	-1.26
ANXA1	-1.30
PLK4	-1.37
ANKRD22	-1.42
LAP3	-1.45
PDGFRB	-1.46
UBE2I	-1.47
IKBKB	-1.48
UBR3	-1.50
PDIA6	-1.60
H2AFX	-1.61
KLF4	-1.64
TBC1D24	-1.71
CDC27	-1.78
CALU	-1.79
CYP1B1	-1.82
CELF2	-1.95
LDHB	-2.00
SOD1	-2.01
MAPK14	-2.01
HSP90AA1	-2.02
NDC80	-2.14
SHC1	-2.15
CASP8	-2.15
CALM3	-2.21
SCD	-2.29
SIRT1	-2.33
GSTO2	-2.36
DIS3L	-2.39
EGF	-2.58
SART1	-2.60
MTOR	-2.62
TTK	-2.74
A2M	-2.76
SLC35G6	-2.98
CDKN2B	-2.99
AURKB	-3.02
DDX21	-3.10
KRT19	-3.13
RHOA	-3.18
PTK2	-3.31
HDAC1	-3.73
ANXA4	-3.89
NR4A2	-4.09
CDC20	-4.21
APOL3	-4.44
APC	-4.54
PPARGC1A	-4.57
LMNB1	-4.93
EZR	-5.15
ANXA2	-5.19
PRKCD	-5.43
PLK3	-5.52
PARP1	-5.83
COMT	-6.01
CDK5	-6.44
CLU	-6.60
IFI30	-6.63
JAG1	-6.79
CCNE1	-6.83
SLC16A3	-6.87
ENO1	-7.09
MCM2	-7.12
FI NA	-8.99
PDZD2	-14.58
BMP2	-23.18

Figure 2. Full spectrum CBD oil exposure results in broad changes in gene expression. A,B,C. Prime PCR Breast Neoplasm Tiers 1,2, and 3 respectively. cDNA from MDAMB-468 cells treated with full spectrum CBD oil or vehicle control were analyzed via qPCR. Validated gene targets were assayed with BioRad Prime PCR arrays. Decreases in gene expression relative to control indicated in green. Increases in gene expression indicated in red. Black indicates no change and grey indicates no data. Fold change indicated is relative to control.

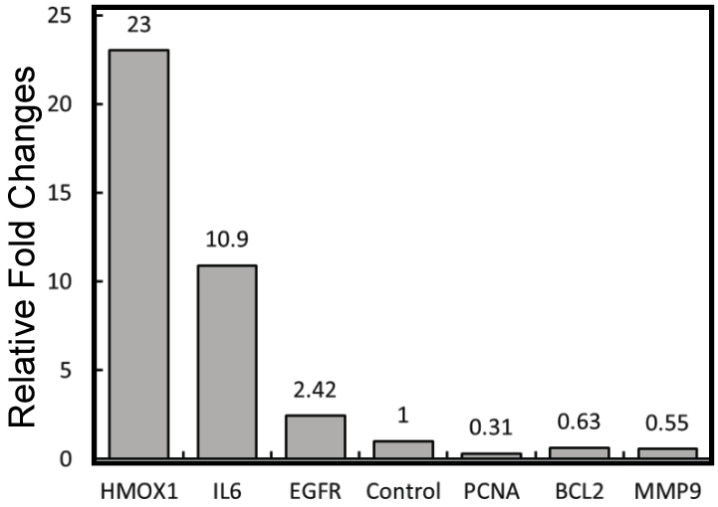

Figure 3. Select changes in gene expression following exposure to full-spectrum CBD oil. Representative Prime PCR targets from each tier shown. Changes in gene expression are relative to control targets set to 1. Select targets were confirmed via qPCR with alternative primer pairs following Prime PCR analysis.

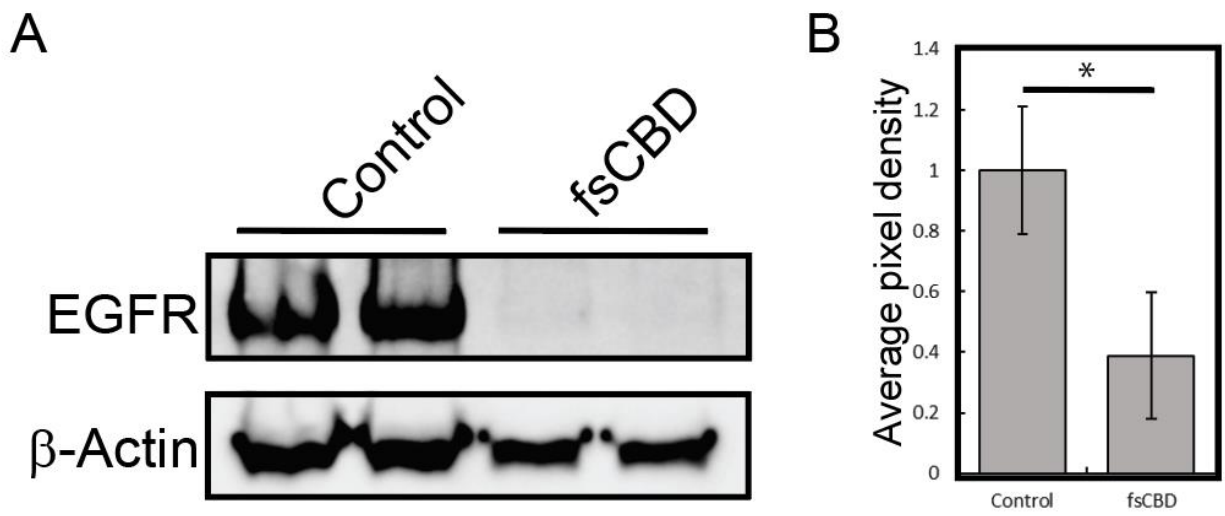

Figure 4. Full spectrum CBD oil reduces EGFR protein *in-vitro*.

MDA MB-468 cells were treated with a 2% solution (V/V) full spectrum CBD oil or control vehicle for 24 hours before immunoblot analysis. A. 40ug of protein were analyzed per sample. Samples loaded in duplicate. B. Pixel density of three independent experiments was analyzed. Error bars are standard deviation of the mean. *p<0.01 Student's T-Test.