Proteomics and Transcriptomics Uncover the Molecular Targets of CBD and THCV in the Sensitization of Doxorubicin Against DOX-resistant MDAMB 231 Xenografts

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Abstract

PURPOSE: To study the Chemosensitization effects of cannabidiol (CBD) and tetrahydrocannabivarin (THCV) in combination with doxorubicin (DOX) against triple negative breast cancers xenografts.

METHODS: The chemo sensitization effect of CBD and THCV in combination with DOX was studied using xenotransplanted DOX resistant MDA-MB-231 cells. After subcutaneous injection of 2.5 million DOX resistant MDA-MB-231 cells in 100 µL matrigel, nude mice were randomized to one of six groups (Control, DOX alone, CBD alone, CBD+DOX, THCV alone and THCV+DOX). In the combination study, CBD (10 mg/kg, i.p.) and THCV (15 mg/kg, i.p.) were given one day before DOX (5 mg/kg, i.p.) to assess the chemo sensitization effect. The treatment was repeated twice a week for 3 weeks until the control group reached 6000 mm3. Using a vernier caliper, the tumor volumes were measured. The animals were euthanized and their blood and tumors collected for further study.

RESULTS: CBD and THCV pre-treatment effectively increased DOX's anticancer potentials, reducing tumor growth and development in mice bearing DOX resistant MDA-MB-231 tumors. Data from RNA sequencing and proteomics revealed that CBD and THCV regulate apoptosis, oxidative stress, and inflammation by targeting the PDL-1 pathway, AMPK pathway, histone proteins, sertonergic pathway, CB1 receptors, and P38-MAPKinase pathway, thereby enhancing the chemosensitization effects of DOX against MDA-MB-231 breast cancers. RT-PCR and westernblot analysis were used to validate the same expression genes and proteins found in RNA sequencing and proteomics. In addition, we discovered significant changes in histone acetylations when CBD/THCV was combined with DOX.

CONCLUSIONS: According to the results of RNA sequencing and proteomic studies, CBD and THCV appear to have a chemosensitization effect on DOX by reversing histone modifications and their downstream effectors.

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Introduction

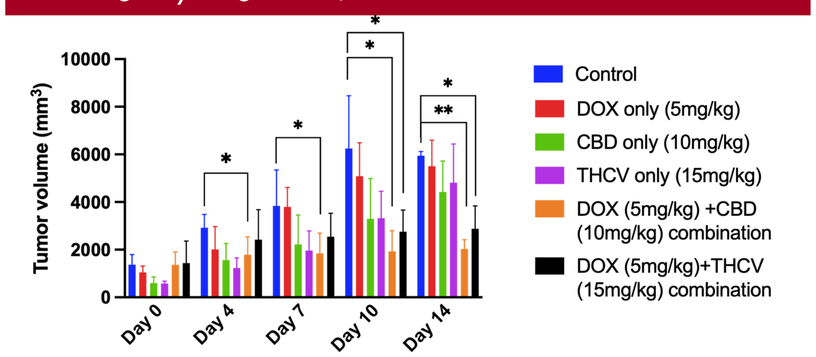
- Breast cancer is the second deadliest cancer in women in the United States, Among breast cancer patients, about 15-20% are diagnosed with TNBC, which is defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)
- Compared with other breast cancer subtypes, TNBC is highly invasive and metastatic and characterized by aggressive clinical course, high relapse rate, and poor overall survival
- Given little efficacy for existing therapeutic regimens against a significant number of TNBC patients and continuously developed resistance of TNBC cells to chemotherapy, novel treatment strategies with significantly enhanced potency and safety are still urgently required to address unmet medical needs
- Among systemic chemotherapeutic agents for TNBC, doxorubicin is one of the most commonly used drugs and resistance to doxorubicin in TNBC is frequently seen and subsequently, the cancer cells develop multi-drug resistant phenotypes
- In recent years, the medicinal use of cannabis and cannabinoids has gained more acceptance in the public domain. Several phytocannabinoids are produced by the plant Cannabis along with terpenes and they target both the endocannabinoid system and other biological pathways
- Presently, cannabinoids, such as dronabinol, nabilone, Epidiolex and others, are approved for the treatment of cancer-related side effects such as nausea and vomiting and also for epileptic seizures
- Studies conducted in our laboratory with CBD in combination with DOX demonstrated that CBD at low doses acted as a chemosensitizer against wild type MDA-MB 231 cells
- Further tumor xenograft studies with MDA-MB 231 cells showed that the combination (CBD+DOX) was able to reduce the tumor burden through decreased expression of proteins involved in inflammation, metastasis and increased expression of proteins involved in apoptosis
- Another minor cannabinoid Δ9-tetrahydrocannabivarin (THCV) has recently shown interest from various investigators for its role in diabetes, pain, weight loss and as an anticancer agent
- In this study for the first time, we have investigated in MDA-MB 231 tumor xenografts, the role and mechanism of action of CBD and THCV in overcoming DOX resistance in TNBC at the molecular level, using RNA seq analysis, proteomics, RT-PCR and western blotting
- Further, the role of various epigenetic markers with cannabinoids in TNBC was also investigated in this study.

Cell Viability of Resistant MDAMB 231 Cells

Drug Solutions	IC50 (µM)
DOX alone	31.00 ± 1.63
CBD alone	2.8 ± 0.13
THCV alone	5.66 ± 0.29
15uM DOX+CBD	1.01 ± 0.18
15uM DOX+THCV	1.79± 0.12
Combination index (CI)	
DOX+CBD	≤ 0.93
DOX+THCV	≤ 0.79
3D DOX res MDA MB- 231 cells culture	
CBD	37.40 µM
THCV	46.23 µM

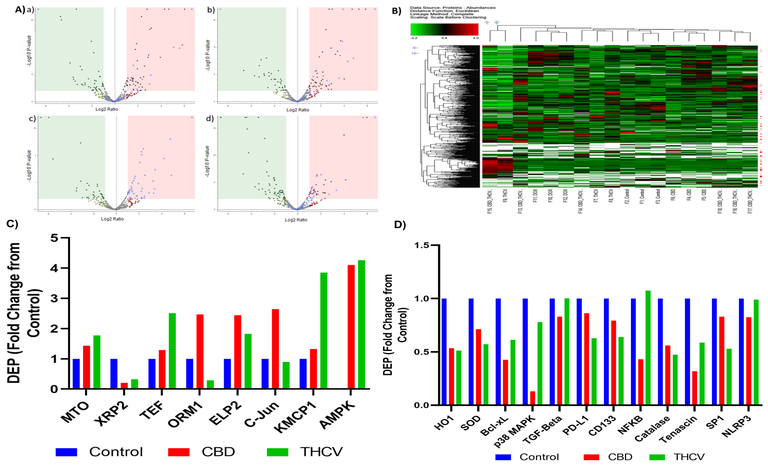
Doxorubicin (DOX), Cannabidiol (CBD), Tetrahydrocannabivarin (THCV), and these cannabinoids combined with DOX in 2D and 3D DOX resistant MDA-MB TNBC cultures are shown in Table 1 (IC50 values and combination index (CI)). The Mean ± SEM (n=3) was used to express the results.

CBD/THCV+DOX reduced the tumor volumes



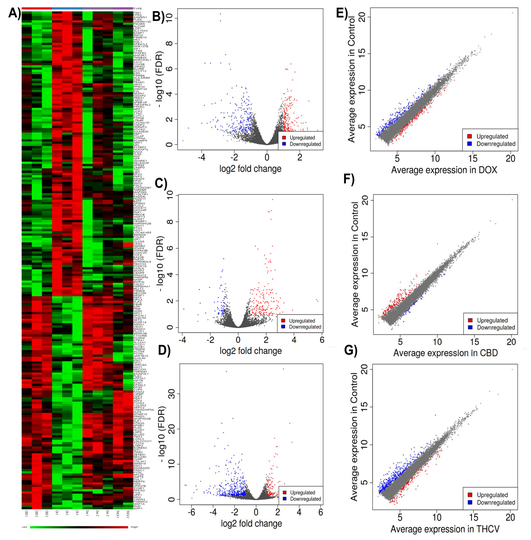
*Bar graph represents the tumor volume data from day 1 to day 14 after initiating the treatment with (Doxorubicin) DOX, Cannabidiol (CBD), Tetrahydrocannabivarin (THCV), DOX+CBD and DOX+ THCV. Values are expressed as Mean ± SEM (n=3). *p<0.05 and **p<0.01 Vs control*

Proteomics Analysis



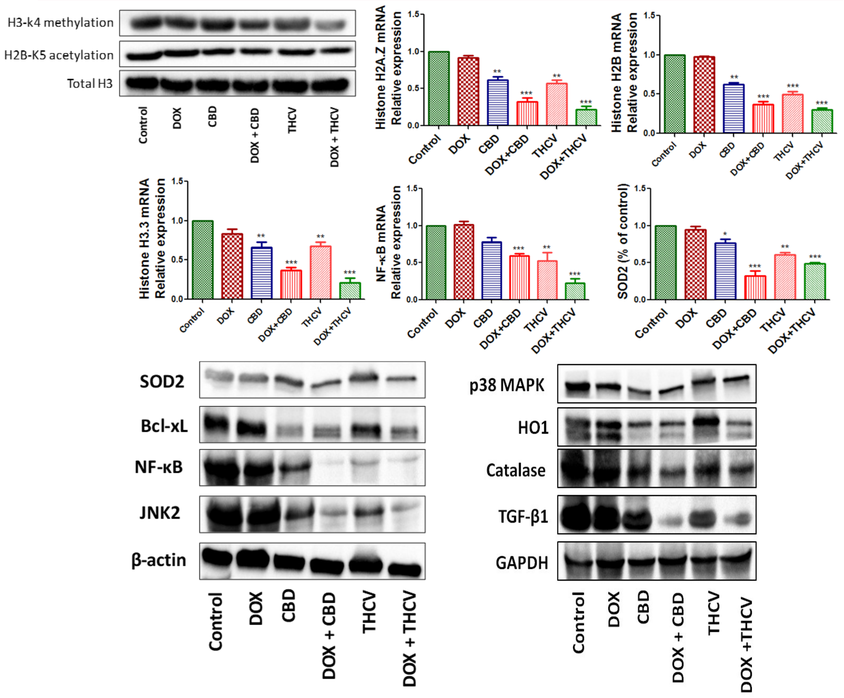
A) Representative Volcano plots of DEPs in between (a) Control and Dox groups, (b) Control and CBD (c) Control and THCV and (d) CBD and THCV (B) Representing illustrations of hierarchical clustering analysis of differentially expressed proteins in control and treatment groups (C) Bar graph representation showing proteins with the largest overall increase in expression and (D) the proteins with the greatest reduction in expression upon treatment are represented.

Transcriptomic Analysis



(a) *Heat map illustrations of hierarchical clustering analysis of differentially expressed mRNA in tissues of control and treated MDA-MB-231 Xenograft mice. Representative volcano plots of differentially expressed genes (DEGs) b) Control between DOX, c) Control between CBD d) Control between THCV. Representative scatter plots of differentially expressed genes (DEGs) e) Control between DOX, f) Control between CBD g) Control between*

Western Blot & RT-PCR Analysis



Conclusion

1.Our in-vitro and in-vivo studies showed that CBD and THCV were effective in overcoming doxorubicin resistance in MDA MB-231 resistant cancer lines

2.In the presence of either CBD or THCV and doxorubicin, MDA-MB 231 cells are rendered inert and eventually die.

3.H3-K4 methylation and H2B-K5 acetylation have been identified as potential resistance markers against doxorubicin in MDA-MB 231 tumors by RNA sequencing, proteomics, western blot, and PCR assays.

4.AMPK, CD133, PDL1, 5HT1A receptors, CB1 receptors, p-38 MAPKinase, and JNK2 were all found to be involved in the progression of resistant triple negative breast cancers.

5.There is still a need for further molecular and knockdown studies to better understand the role of these molecular targets in the resistance of MDA MB-231 breast cancer cell lines to doxorubicin