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Combined CB2 Receptor Agonist and Photodynamic Therapy Synergistically Inhibit Tumor Growth in Triple Negative Breast Cancer

Jiliang Zhang^{#a}, Shaojuan Zhang^{#b}, Yang Liu^c, Meng Su^c, Xiaoxi Ling^b, Funan Liu^d, Yinghui Ge^{*,a}, and Mingfeng Bai^{*,b,e,g}

^aDepartment of Radiology, Henan provincial people's hospital, 7# weiwu road, Zhengzhou, Henan, China, 450003

^bDepartment of Radiology, University of Pittsburgh, 100 Technology Drive, Pittsburgh, PA 15219, USA

^cVanderbilt University Institute of Imaging Sciences, Nashville, TN 37232, USA

^dDepartment of Surgical Oncology and General Surgery, First Affiliated Hospital of China Medical University, Shenyang, China, 110001

^eDepartment of Medicine, University of Pittsburgh, 3501 Fifth Ave, Pittsburgh, PA 15213, USA

^fDepartment of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15261, USA

^gUniversity of Pittsburgh Cancer Institute, Pittsburgh, PA 15232, USA

[#] These authors contributed equally to this work.

Abstract

Triple negative breast cancer (TNBC) is the deadliest form of breast cancer because compared with other types of breast cancer, it is more aggressive, diagnosed at later stage and more likely to develop recurrence. Many patients do not experience adequate tumor control after current clinical treatments involving surgical removal, chemotherapy and/or radiotherapy, leading to disease progression and significantly decreased quality of life. Here we report a new combinatory therapy strategy involving cannabinoid-based medicine and photodynamic therapy (PDT) for the treatment of TNBC. This combinatory therapy targets two proteins upregulated in TNBC: the cannabinoid CB2 receptor (CB₂R, a G-protein coupled receptor) and translocator protein (TSPO, a mitochondria membrane receptor). We found that the combined CB₂R agonist and TSPO-PDT treatment resulted in synergistic inhibition in TNBC cell and tumor growth. This combinatory

*To whom Correspondence should be addressed, Mingfeng Bai, Postal Address: AAA-3105 MCN, 1161 21st Ave South, Nashville, TN 37232, Telephone: +1-615-343-7753, mingfeng.bai@vanderbilt.edu.

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Disclosures

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therapy approach provides new opportunities to treat TNBC with high efficacy. In addition, this study provides new evidence on the therapeutic potential of CB₂R agonists for cancer.

Keywords

Photodynamic therapy; CB₂ receptor; synergistic; combination therapy; triple negative breast cancer; cannabinoid, agonist

1. Introduction

Cannabis (marijuana) has been exploited for medicinal and spiritual purposes for thousands of years. However, the discovery of Δ^9 -tetrahydrocannabinol (THC) as the principle bioactive component of marijuana only occurred in 1964(1), and the identification of CB₁ and CB₂ receptors as part of human endocannabinoid system did not occur until 1991(2) and 1993(3), respectively. We now know that the biologic effects of THC are mediated primarily by its binding to these two cannabinoid receptors, although additional receptors and non-receptor interactions may also occur(4). Both of these receptors belong to the G protein-coupled receptor (GPCR) family. Under homeostatic conditions, CB₁R expression predominates in the brain and CB₂R is mainly a peripheral receptor abundantly expressed by immune cells(5, 6), and to a lesser extent by other tissues and organs(7). Activation of CB₁R drives the well-known psychotropic effects of marijuana; thus, the scientific fields and pharmaceutical industries have been increasingly interested in understanding the therapeutic potential of cannabinoids that selectively bind to CB₂R without altering memory and perception. This interest stems from the fact that researchers have found up-regulated CB₂R expression in a number of conditions and have shown that CB₂R plays an important role in the regulation of these diseases, including inflammation(8, 9), pain(10), osteoporosis(11), autoimmune diseases(12), addiction(13, 14), psychiatric disorders(15–17), diabetes(18–20), cardiovascular disorders(21), cancers and neurodegenerative diseases(5, 22).

Recent studies have shown that CB₂R plays a fundamental role in tumorigenesis and that CB₂R agonists may represent a promising approach for cancer treatments(23, 24). Specifically, studies demonstrated anti-tumor effect of CB₂R agonists on various types of cancers, such as breast(25–28), prostate(29, 30), glioma(31), colon(32), lung(33), thyroid(34), lymphoma(35), skin(36), pancreas(37), and liver(38) cancers. In particular, due to the large impact and current challenge of breast cancer treatment, great efforts have been invested in cannabinoid therapy of this cancer type. CB₂R agonists have shown therapeutic potential in treating hormone [estrogen receptor (ER) and/or progesterone receptor (PR)] sensitive, human epidermal growth factor receptor 2 (HER-2) positive, and triple negative (negative status of ER, PR and HER-2) breast cancer (TNBC) cells(26). In addition to these encouraging *in vitro* results, a CB₂R agonist, JWH-133, has also been shown to inhibit the growth and lung metastasis of triple negative MDA-MB-231 breast tumor in mouse tumor models(25).

Despite the promise, the field of CB₂R-mediated breast cancer therapy is still in its infancy. Only a few studies have been reported with limited therapeutic efficacy. For example, 42% of breast tumor growth was observed even after daily peritumoral injection of 5mg/kg body

weight of JWH-133 for 4 weeks(25). In addition, although most reports suggest anti-tumor actions of cannabinoids, pro-tumor effects of cannabinoid therapy have also been reported(39). Therefore, there is need to further explore the therapeutic potential of CB₂R agonists in TNBC treatment and develop new treatment strategies to improve the therapeutic efficacy. The purpose of this study is to develop a new combinatory therapeutic approach (CB₂R agonists + photodynamic therapy) to treat TNBC with high efficacy and further evaluate the potential of CB₂R-mediated breast cancer therapy *in vitro* and *in vivo*. As a minimally invasive and highly controllable therapeutic procedure, photodynamic therapy (PDT) has attracted increasing attention for cancer treatment. In our recent study, we developed a photosensitizer, IR700DX-6T, which targets the 18 kDa translocator protein (TSPO), previously termed the peripheral benzodiazepine receptor (PBR)(39). Several studies have shown dramatically increased density of TSPO in breast carcinomas as compared with normal breast tissues(40–42). In addition, TSPO expression is much higher in highly aggressive TNBC cells, such as MDA-MB-231, as compared with non-aggressive ones, such as MCF-7 (~20 fold more TSPO mRNA in MDA-MB-231 than in MCF-7)(40). Consequently, TSPO serves as a promising therapeutic target for TNBC. Our data showed that TSPO-PDT using IR700DX-6T significantly inhibited the growth of MDA-MB-231 TNBC cells and tumors in a target-specific manner(39). Encouraged by the success of TSPO-PDT, we set out to combine cannabinoid therapy with TSPO-PDT to treat TNBC. Our results suggest that cannabinoid therapy using CB₂R agonists may have great potential in improving therapeutic outcome of TNBC.

2. Materials and methods

2.1. General

The solvents used are of ACS or HPLC grade. The photosensitizer, IR700DX-NHS ester, was purchased from LI-COR Bioscience (Lincoln, NE). JWH-133 was purchased from Cayman chemical (Ann Arbor, MI). The following instruments, supplies and assay kits were used for *in vitro* and *in vivo* studies: Synergy™ H4 Hybrid Multi-Mode Microplate Reader (BioTek, Winooski, VT), Zeiss Axio Observer fluorescence microscopy system (Zeiss, Jena, Germany), 96-well optical black plates (Fisher Scientific, Pittsburgh, PA), CellTiter-Glo Luminescent Cell Viability Assay kit (Promega, Madison, WI), Agilent Technology 6130 LCMS (Santa Clara, CA) and IVIS Lumina XR *in vivo* imaging system (PerkinElmer, Waltham, MA). MDA-MB-231 human breast cancer cells were purchased from ATCC (Catalog # HTB-26).

2.2. Synthesis of IR700DX-6T

IR700DX-6T was synthesized by coupling IR700DX-NHS ester with a conjugable TSPO ligand, 6-TSPOMBb732, using our previously reported method(43).

2.3. Cell lines and culture conditions

MDA-MB-231 human breast cancer cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich, St. Louis, MO) containing 10% fetal bovine serum (FBS, Fisher Scientific, Pittsburgh, PA), and 1% Penicillin-Streptomycin-Glutamine (Life Technology, Carlsbad, CA). 4T1 murine breast cancer cells were cultured in RPMI 1640

Medium (Life Technology, Carlsbad, CA) containing 10% fetal bovine serum, and 1% Penicillin-Streptomycin-Glutamine. Cells were incubated in a water jacketed incubator (37 °C, 5% CO₂).

2.4. Singlet oxygen production

A stock solution of 500 µM singlet oxygen sensor green (SOSG) was prepared by dissolving 10 µg of SOSG in methanol (330 µL). 4 µL of the stock solution was then added to 200 µL of 40 µM JWH-133, 5 µM IR700DX-6T, or 40 µM JWH-133 + 5 µM IR700DX-6T in DMEM. Another 4 µL of the SOSG stock solution was added to 200 µL DMEM as the vehicle control. Samples were then placed under a 690 nm LED light for 30 min with a power of 30 mW/cm² before fluorescence measurement. Excitation wavelength was 480 nm. Spectra were recorded from 510 to 700 nm.

2.5. In vitro phototherapy study

To examine the combined therapeutic effect of IR700DX-6T-PDT and JWH-133 *in vitro*, MDA-MB-231 or 4T1 cells were seeded into 96-well plates and incubated for 24 h prior to treatment. Cells were divided to 4 groups as follows: (1) Vehicle as non-treatment control group; (2) IR700DX-6T + light irradiation; (3) JWH-133 + light irradiation; (4) Combined IR700DX-6T and JWH-133 + light irradiation. For each treatment group, cells were incubated with IR700DX-6T, JWH-133 or both at 37 °C for 24 h. For study with MDA-MB-231 cells, single dose of IR700DX-6T was 0, 1.0, 1.5, 2.0, 2.5, and 3.0 µM; single dose of JWH-133 was 0, 8, 12, 16, 20, 24 µM and combined doses of IR700DX-6T and JWH-133 (1:8 ratio) were IR700DX-6T (1.0, 1.25, 1.5, 1.75, 2.0 µM) and JWH-133 (8.0, 10, 12, 14, 16 µM). For study with 4T1 cells, single dose of IR700DX-6T was 0, 1.0, 1.5, 2.0, 2.5, and 3.0 µM; single dose of JWH-133 was 0, 8, 12, 16, 20, 24 µM; and combined doses of IR700DX-6T and JWH-133 (1:8 ratio) were IR700DX-6T (1.0, 1.5, 2.0, 2.5, 3.0 µM) and JWH-133 (8.0, 12, 16, 20, 24 µM). Cells were then washed once to remove the unbound chemicals with cell culture medium. Subsequently, cells were irradiated with LED light (L690-66-60, Marubeni America Co., New York, NY) at wavelengths of 670–710 nm and a power density of 30 mW/cm² for 30 min. Then cells were returned to incubator for an additional 24 h prior to cell viability assay. Cell viability was determined by CellTiter-Glo[®] Luminescent Cell Viability Assay per the manufacturer's instructions. The luminescent intensity was directly proportional to the amount of remaining viable cells. Cell death rates were determined by one minus recorded luminescent intensity in each group over that of the vehicle group times one hundred percent.

2.6 Combination index calculations

The median-effect analysis of Chou-Talalay Method was used to determine additive, synergistic or antagonistic effect for combination therapy with JWH-133 and IR700DX-6T-PDT. *In vitro* therapeutic effect data were used to calculate combination index (CI) via CompuSyn (Combosyn, Inc., Paramus, NJ) software.

$$CI = \frac{C_{A,x}}{IC_{x,A}} + \frac{C_{B,x}}{IC_{x,B}}$$

$C_{A,x}$ and $C_{B,x}$ are the concentrations of drug A and drug B used in combination to achieve x % drug effect. $IC_{x,A}$ and $IC_{x,B}$ are the concentrations for single agents to achieve the same effect(44). CI values <1 , $=1$, and >1 indicate synergistic, additive, and antagonistic effects, respectively.

The isobologram analysis, similar to CI analysis, evaluates the interaction of two drugs. First, when drug A or B is used alone, the concentrations required to produce a defined single-agent effect (*e.g.*, IC50) are placed on the x and y axes in a two-coordinate plot, corresponding to $(C_A, 0)$ and $(0, C_B)$, respectively. These two points are then connected and the line is defined as the line of additivity. Second, when the two drugs are used together, concentrations of them needed to provide the same effect, denoted as (c_A, c_B) , are placed in the same plot. Synergy, additivity, or antagonism are indicated when (c_A, c_B) is located on the lower left, on, or on the upper right of the line of additivity, respectively.

2.7 JWH-133 photostability study.

JWH-133 solution (3 μ L of 10 mM in DMSO) before and after 690 nm light irradiation (30 min, 30 mW/cm²) was injected into LC/MS system. Buffer A: 0.1% TFA in Water, buffer B: acetonitrile. Elution method: 40% B to 95% B in 1.5 min. Liquid chromatography was monitored under 254 nm. Areas of the peaks corresponding to JWH-133 (based on mass spectrum) were integrated to quantify the photostability of JWH-133.

2.8 In vivo combination PDT efficacy.

The animal studies have been approved by the University of Pittsburgh Institutional Animal Care and Use Committee (IACUC). Female athymic nude mice at 6–8 weeks old were purchased from the Charles River Laboratory (Wilmington, MA). 5×10^6 MDA-MB-231 cells were injected subcutaneously into the left flank of the mice. Mice were anesthetized with a 2.5% isoflurane/oxygen gas mixture during treatments. The mice were euthanized with cervical dissection under anesthesia once the tumor diameter reached 15 mm at any direction.

In vivo PDT experiments were carried out approximately 7 days after cell injection. Mice with approximate tumor sizes of 100 mm³ were selected. Tumor-bearing mice were randomized into 4 groups ($n = 3$ per group) with the following treatments: (1) Vehicle + light irradiation, (2) IR700DX-6T + light irradiation: 5 nmol of IR700DX-6T i.v. injection, followed by light irradiation at 2 h post injection, (3) JWH-133 + light irradiation: 40 nmol JWH-133 i.v. injection, followed by light irradiation at 2 h post injection, and (4) Combined treatment: (5 nmol of IR700DX-6T + 40 nmol of JWH-133) i.v. injection, followed by light irradiation at 2 h post injection. Light irradiation at the tumor area was given by an LED light at a power density of 50 mW/cm² for 15 min (45 J/cm²). The above treatments were performed only once. The tumor sizes were measured daily by a caliper until termination point and the volume was calculated as $(\text{tumor length}) \times (\text{tumor width})^2 \div 2$.

2.9. Data Processing and Statistical analysis

All of the data given in this study are the mean $\pm$ SEM (standard error of the mean) of n independent measurements ($n = 4$ for *in vitro* study, and $n = 3$ for *in vivo* study). *In vitro* statistical analyses were performed using the Mann-Whitney test. *In vivo* statistical analyses were performed using the one-way ANOVA method, with p values < 0.05 considered statistically significant. The analyses were performed using SPSS11.0 (SPSS Inc., Chicago, IL).

3. Results

3.1 Synergistic cytotoxicity of IR700DX-6T-PDT and JWH-133 in MDA-MB-231 cells

We first evaluated the singlet oxygen production of IR700DX-6T + JWH-133 as compared with IR700DX-6T. Upon light irradiation, the mixed IR700DX-6T and JWH-133 sample produced similar amount of singlet oxygen as IR700DX-6T (Figure S1). We also evaluated the photostability of JWH-133 by analyzing the amount of the compound before and after light irradiation (690 nm, 30 min, 30 mW/cm²) using liquid chromatography mass spectrum (LC/MS). As shown in Figure S2, negligible change (integrated area of 152, before irradiation, versus 154, after irradiation) of eluted JWH-133 was observed, suggesting that JWH-133 is photostable under our treatment condition.

We then studied the effect of single treatment of CB₂R agonist JWH-133 or IR700DX-6T-PDT in MDA-MB-231 cells. As shown in Figure 1A–B, after treatment with JWH-133 or IR700DX-6T-PDT alone, cell death increased in a concentration-dependent manner. To evaluate whether the combined treatment could enhance the therapeutic effect, MDA-MB-231 cells were divided into 3 treatment groups: (1) JWH-133; (2) IR700DX-6T-PDT; and (3) combined JWH-133 and IR700DX-6T-PDT. Cytotoxicity induced by the above treatments was subsequently evaluated and compared. As shown in Figure 1C, greater cell death was observed with the combined treatment. For example, 8 μ M of JWH-133 and TSPO-PDT with 1 μ M of IR700DX-6T alone induced only $7.6\% \pm 2.9\%$ and $28.6\% \pm 1.7\%$ cell death, respectively, and the combined therapy caused as high as $52.3\% \pm 0.6\%$ cell death. Similarly, 10 μ M of JWH-133 and TSPO-PDT with 1.25 μ M of IR700DX-6T alone induced $9.0\% \pm 1.8\%$ and $34.4\% \pm 1.4\%$ cell death, respectively, and the combined treatment resulted in $60.5\% \pm 0.9\%$ cell death.

To quantify the synergistic effect of combinatory treatment with JWH-133 and IR700DX-6T-PDT, We calculated CI values based on the Chou and Talalay's median-effect equation(45). As shown in Table 1 and Figure 2A (Fa-CI plot), the CI values in all combined treatments are less than 1, indicating that the combination of JWH-133 and IR700DX-6T-PDT had synergistic cytotoxic effects on MDA-MB-231 cells at the indicated concentrations. Additionally, according to the isobologram analysis (Figure 2B), the equipotent doses of IC₅₀ and IC₇₅ were plotted at the left lower area of the corresponding line of additivity (IC₅₀ and IC₇₅), suggesting synergistic effect. In observation of the relatively modest synergistic effect (CI values close to 1) and to provide further evidence on the synergy between JWH-133 and IR700DX-6T-PDT, we evaluated the therapeutic effect using a murine TNBC cell line, 4T1. As shown in Table 2 and Figure S3, the CI values in all

combined treatments are also below 1. These combined data suggest that JWH-133 and IR700DX-6T-PDT synergistically caused TNBC cell death.

3.2 In vivo therapeutic effect

We investigated the *in vivo* tumor inhibition effect caused by JWH-133, TSPO-PDT or combinatory treatment using a subcutaneous MDA-MB-231 xenograft mouse tumor model (Figure 3A–B). Animals were randomized into 4 groups with the following treatments: (1) Vehicle + light irradiation, (2) JWH-133 + light irradiation, (3) IR700DX-6T + light irradiation, and (4) Combined JWH-133 and IR700DX-6T + light irradiation. To account for potential light irradiation effect, all animals were subject to light treatment. Tumor volumes were then measured at 5 days after treatment to quantify the therapeutic outcome. We found that the combined treatment significantly inhibited tumor growth as compared to the vehicle control treatment (average tumor volume $381.0 \pm 118.5 \text{ mm}^3$ vs $1103.3 \pm 196.9 \text{ mm}^3$, $p < 0.05$). Additionally, combination therapy showed greater tumor inhibition effect than single treatment of JWH-133 ($381.0 \pm 118.5 \text{ mm}^3$ vs 969.3 ± 227.3 , $p < 0.05$), or IR700DX-6T-PDT ($381.0 \pm 118.5 \text{ mm}^3$ vs $951.8 \pm 81.4 \text{ mm}^3$, $p < 0.05$).

The survival of the tumor-bearing mice (Figure 3C) was also compared among all the groups. The mice were euthanized once the tumor diameter reached 15 mm at any direction. Mice in the vehicle control group quickly reached the termination point owing to aggressive tumor growth (median survival time = 5 days). Single treatment of IR700DX-6T-PDT or JWH-133 did not significantly prolong the survival time (median survival time = 5 and 6.5 days). In contrast, mice received combined JWH-133 and IR700DX-6T upon light irradiation exhibited the longest survival time (median survival time = 8 days). These data indicate that combined JWH-133 and TSPO-PDT treatment led to the greatest therapeutic efficacy in TNBC tumors.

Discussion

TNBC accounts for about 15% of all breast cancer cases(46). Compared with other types of breast cancer, TNBC have a poorer survival rate because TNBC is more aggressive, diagnosed at later stage and more likely to recur(46). Unfortunately, although ER, PR and/or HER-2 positive breast cancer patients can benefit from targeted therapy, no targeted therapy for TNBC has been established for clinical use(47). Current clinical treatment of TNBC relies mainly on resection, chemotherapy and/or radiotherapy. However, residual tumors are often left behind after surgery and tumors frequently develop resistance to chemotherapy and radiation. Therefore, new strategies for TNBC treatment with high efficacy are urgently needed.

CB₂R is emerging as a new target for cancer treatment. In particular, several studies have suggested therapeutic value of CB₂R agonists in breast cancer, especially TNBC, through CB₂R activation(25–28). Several human TNBC cell lines, such as MDA-MB-231, have been shown to express CB₂R at higher levels than CB₁R(48). In addition, no significant CB₂R expression was detected in normal breast tissue and treatment of CB₂R agonists has been shown to have no effect on normal breast cancer cells and tissue(28, 49). Moreover, unlike CB₁R, CB₂R activation does not trigger un-desirable psychoactive effect. As such,

cannabinoid therapy using CB₂R agonists may offer new hope for TNBC. However, limited therapeutic outcome of CB₂R agonists in TNBC treatment has been reported(25).

Cancer is a complex disease characterized by deregulation of diverse cellular systems. Combined treatment that targets multiple signaling transduction pathways is believed to enhance therapeutic efficacy and reduce resistance developed by cancer cells(45, 50). Recent studies suggest that combination therapy involving PDT and other treatments, such as chemotherapy, immunotherapy, anti-oxidants, angiogenesis inhibitors, and radiotherapy, is a promising strategy to improve therapeutic efficacy(50, 51). For example, the combination of chemotherapy and PDT has been shown to promote synergism between distinct cell killing pathways, and therefore greatly enhance the anticancer efficacy(52, 53). We expected that by combining CB₂R activation and mitochondria damage via TSPO-PDT, desirable synergistic effect could be achieved. To our best knowledge, this is the first combination therapy study involving targeted PDT and CB₂R agonist. It is noteworthy that we recently reported a CB₂R-targeted PDT agent, IR700DX-mbc94 (54–56). The CB₂R-targeting molecule, mbc94, is conjugated to the photosensitizer, IR700DX. In addition, the structure of mbc94 is based on a CB₂R antagonist, SR144528. Therefore, the mbc94 component is expected to have only targeting functionality without therapeutic capability.

To account for the relatively low expression level of CB₂R in MDA-MB-231 human TNBC cells(28, 49), we used a ratio of 1:8 IR700DX-6T/JWH-133 in combined treatment *in vitro* and *in vivo*. All therapeutic results were compared with those from single treatment and untreated group. Both single and combined treatments caused therapeutic effect in a concentration-dependent manner. As expected, the combination of TSPO-PDT with JWH-133 led to synergistic inhibition of MDA-MB-231 cell and tumor growth.

The present study was focused on assessing the combinatory therapy effect of TSPO-PDT and JWH-133. The targeting effects of IR700DX-6T and JWH-133 have been previously reported and therefore were not investigated. Specifically, IR700DX-6T induced apoptotic cell death in TSPO-positive MDA-MB-231 but not TSPO-negative MCF-7 breast cancer cells(43). *In vivo* PDT study suggested that IR700DX-6T-mediated PDT inhibited the growth of MDA-MB-231 tumors at much greater extent than non-targeted IR700DX-PDT, and such therapeutic effect was effectively suppressed by a blocking agent(43). Similarly, JWH-133 has been shown to inhibit the growth of MDA-MB-231 cells and tumors, and such effects were reversed by a CB₂R antagonist(25).

Therapeutic synergy associated with combination therapy has been reported to be promoted through multi-target mechanisms (57). Although comprehensive mechanistic study is beyond the scope of this study, we speculate that the synergism between TSPO-PDT and JWH-133 is due to the activation of multiple signaling and cell death pathways. Specifically, JWH-133 was reported to inhibit breast tumor growth through regulation of COX-2 pathway(48), whereas TSPO-PDT directly introduces large amount of ROS to tumor mitochondria, resulting in effective apoptotic cell death(43, 58). Our data also showed that the synergistic effect between TSPO-PDT and JWH-133 is relatively modest (most CI values are close to 1). The relatively low contribution of JWH-133 to the combined treatment, as shown in Figure 1B and 1C, may account for the modest synergistic effect.

Although increasing the dose of JWH-133 is expected to improve the therapeutic effect, high concentrations of JWH-133 may also cause significant side effects. Delivery of large amount of JWH-133 to the tumor site using nanocarriers, or development of JWH-133 prodrugs that can be activated in the tumor, may further improve the synergy with TSPO-PDT treatment.

It is noteworthy that JWH-133 caused limited therapeutic effect when used alone. Although previous study showed that 10 μ M of JWH-133 caused roughly 40% death of MDA-MB-231 cells, we did not observe similar effect until 24 μ M of JWH-133 was used (Figure 1B).

Unlike PDT treatment using IR700DX-6T, JWH-133 was not able to cause dramatic cells death or tumor inhibition. Consistent with other reports(26, 59, 60), our results suggest that the therapeutic action of CB₂R agonists may be the best implemented when combined with other therapeutic methods. Regardless, compelling evidence has shown the anti-tumor activity of CB₂R agonists. Additionally, an important feature of cannabinoid-based medicines for clinical use is the low toxicity, as they have been proven to be safe in patients in many clinical trials(59). Therefore, CB₂R agonists could be promising therapeutics for cancers, such as TNBC.

Conclusions

In this study, we investigate the potential of combined cannabinoid therapy and PDT in TNBC treatment. Through targeting two cellular receptors associated with TNBC, TSPO and CB₂R, synergistic effects were observed in both *in vitro* and *in vivo* experiments, and the median survival time of tumor-bearing animals was greatly extended. These encouraging results suggest that CB₂R agonists may have great promise in enhancing TNBC therapeutic efficacy. Further, this strategy may be widely applied to treat various cancer types and we are looking forward to the prospect of advancing CB₂R-mediated therapy for cancer treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- A new combinatory therapy strategy for the treatment of triple negative breast cancer
- Synergistic CB₂R agonist and PDT treatment inhibited tumor growth
- Cannabinoid-based medicine has potential in cancer therapy

Figure 1. Combined IR700DX-6T-PDT and JWH-133 induced greater cell death than single treatment.

MDA-MB-231 cells were treated with (A) IR700DX-6T-PDT, (B) JWH-133 or (C) combined

IR700DX-6T-PDT and JWH-133. * indicates $p < 0.05$.

Figure 2. Synergistic effect of combination treatment using IR700DX-6T-PDT and JWH-133 on MDA-MB-231 cells.

(A) Chou-Talalay plot to determine the synergistic effect of combined TSPO-PDT and CB₂R agonist. (B) Isobologram of combined IR700DX-6T-PDT and JWH-133.

A **B** **C**

Figure 3. In vivo combination PDT treatment using IR700DX-6T and JWH-133 on MDA-MB-231 tumor bearing mice.

(A) White light images of representative mice in each group at 5 days post-PDT treatment.

(B) Tumor growth was compared among groups at 5 days post-PDT treatment. (C) Survival

curves compared among groups. * indicates $p < 0.05$ and ** indicates $p < 0.01$.

Table 1.

Synergistic effect of combined PDT treatment of IR700DX-6T and JWH-133 on MDA-MB-231 cells.

JWH-133 (μM)	IR700DX-6T (μM)	CI
8	1.0	0.81
10	1.25	0.87
12	1.5	0.93
14	1.75	0.87
16	2.0	0.94

Table 2.

Synergistic effect of combined PDT treatment of IR700DX-6T and JWH-133 on 4T1 cells.

JWH-133 (μM)	IR700DX-6T (μM)	CI
8	1.0	0.69
12	1.5	0.66
16	2.0	0.79
20	2.5	0.80
24	3.0	0.84