Advances in Cancer Biology - Metastasis 5 (2022) 100043

Contents lists available at ScienceDirect

Advances in Cancer Biology - Metastasis

journal homepage: www.journals.elsevier.com/advances-in-cancer-biology-metastasis

Synthetic cannabinoid WIN 55,212–2 inhibits growth and induces cell death of oral and pancreatic stem-like/poorly differentiated tumor cells

Meng-Wei Ko [a,f], Barbara Breznik [a,b,f], Emanuela Senjor [a,c,d,f], Anahid Jewett [a,e,f,*]

[a] Division of Oral Biology and Oral Medicine, UCLA, Los Angeles, CA, USA
[b] Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, Slovenia
[c] Department of Biotechnology, Jožef Stefan Institute, Ljubljana, Slovenia
[d] Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia
[e] The Jonsson Comprehensive Cancer Center, UCLA School of Dentistry and Medicine, Los Angeles, CA, USA
[f] The Jane and Jerry Weintraub Center for Reconstructive Biotechnology, UCLA, Los Angeles, CA, USA

ARTICLE INFO

Keywords:
WIN 55,212–2
Cancer stem cells (CSCs)
Differentiation
Cell death

ABSTRACT

We report here that synthetic cannabinoid WIN 55,212–2 inhibits tumor cell proliferation and induces cell death of oral and pancreatic tumor cells, and the effect is much more pronounced on stem-like/poorly differentiated OSCSCs and MP2 cells when compared to well-differentiated OSCCs, and PL-12 tumor cells. In addition, WIN 55,212-2 decreases cell surface expression of CD44, CD54, MHC class I and PD-L1 on oral and pancreatic tumor cells with the exception of PD-L1 expression on well-differentiated PL-12 pancreatic tumor cells which exhibits an increase in the expression rather than a decrease. Overall, we demonstrate that WIN 55,212-2 has an increased targeting activity against cancer stem cells/poorly differentiated oral and pancreatic tumor cells when compared to well-differentiated tumor cells, and furthermore, such differences in function do not correlate with the levels of CB1 and CB2 receptor expression on tumor cells, suggesting it's function either through post-receptor mediated activation and/or yet-to-be identified novel receptors. Intraperitoneal (IP) delivery of WIN 55-212-2 in humanized BLT mice is found to impart an activating potential for NK cells demonstrating increased NK cell mediated cytotoxicity and secretion of IFN-γ in our preliminary experiments. These results not only suggest a direct targeting of CSCs/poorly differentiated tumors by WIN 55-212-2 but also by indirect targeting of such tumors through the activation and increased functions of NK cells.

1. Introduction

Cannabinoid-based drugs have been used as palliative treatments along with conventional therapy for amelioration of side effects of radio- and chemotherapy to reduce nausea and stimulate appetite in cancer patients [1]. Cannabinoids were shown to act through activating cannabinoid receptors, CB1 and CB2. Both of these receptors were shown to be increased on tumor cells of multiple origin, including prostate, glioblastoma, hepatocarcinoma [2], breast [3], and non-small cell lung cancer [4]. Components of endocannabinoid system has been shown to have anti-tumor effects by inhibiting the proliferation and inducing cell death through apoptosis [5]. WIN 55,212–2, a potent cannabinoid receptor agonist with a chemical structure shown in Fig. 1A, was previously reported to mediate anti-tumor effect through inducing caspase-independent apoptosis, in addition to inhibiting migration and invasion of tumors in several studies, such as glioblastoma [6], renal cell carcinoma [7], hepatocellular carcinoma [8], osteosarcoma [9], tumorigenic epidermal tumors [10], prostate tumors [11], human Kaposi's sarcoma tumors [12], mantle cell lymphoma [13], melanoma [14] and breast cancers [15–17]. It was also reported that WIN 55,212-2 synergistically increased the effects of radiotherapy in breast cancer cell lines but not in normal breast epithelium, whereas other cannabinoids such as CBD, nabilone and THC failed to enhance anti-proliferative effects of radiation [15]. Furthermore, WIN 55,212–2 was shown to reduce tumor burden, lung metastasis and tumor induced angiogenesis *in vivo* in mouse models of breast cancer [3], non-small cell lung cancer [4] and nonmelanoma skin cancer [10].

Tumors contain a population expressing CD133, ALDH and CD44 phenotype, with a specific genetic signature known as cancer stem-like cells, which sustain tumor growth because of self-renewal capacity and high rates of proliferation [18–21]. In addition, CSCs/undifferentiated

* Corresponding author. UCLA School of Dentistry, 10833 Le Conte Ave., Los Angeles, CA, 90095, USA.
E-mail address: ajewett@ucla.edu (A. Jewett).

https://doi.org/10.1016/j.adcanc.2022.100043
Received 12 January 2022; Received in revised form 23 April 2022; Accepted 25 April 2022
Available online 19 May 2022

Abbreviations

CSC	cancer stem cells
MHC class I	Major Histocompatibility Complex Class I
PD-L1	Program Death Ligand 1
CBD	Cannabidiol
THC	Tetrahydrocannabinol
PD-1	Program Death-1
EDTA	Ethylenediamine tetraacetic acid
OSCC	Oral Squamous Carcinoma Cells
OSCSC	Oral Squamous Carcinoma Stem Cells
CDDP	*cis*-diamminedichloroplatinum(II)
MP2	Mia-PaCa2
PI	Propidium Iodide

or poorly differentiated tumors are known to have aggressive phenotype with a potential to give rise to metastatic growth after implantation of few tumor cells in mice [19,22–24]. We, as well as others, have previously demonstrated that NK cells target both normal healthy [25–27], as well as a number of different CSCs/poorly differentiated tumors [28,29]. Indeed, it is possible that NK cells are important in selecting and differentiation of many different types of stem cells, including adipose tissue stem cells [30,31]. In addition, our recent work based on several different CSCs/undifferentiated tumors such as pancreatic, oral, melanoma, glioblastoma and lung has established NK cells as the major immune effectors responsible for targeting and differentiation of these tumors [32–34]. Indeed, we have recently found that all of the generated invasive clones of breast tumors with different genetic/epigenetic and phenotypic abnormalities were not only enriched by CSCs but also they were highly susceptible to NK cell mediated cytotoxicity, and increased secretion of IFN-γ, as compared to their parental lines [35]. In addition, in all different types of CSCs which we have examined and reported previously, a generalized profile of surface receptor expression was identified, demonstrating a lack or a decrease in the levels of MHC-class I, CD54 and PD-L1 and an increase in CD44 expression [33,34]. We have used these surface markers to successfully differentiate between a number of distinct stem-like/poorly differentiated and well differentiated tumor types [32,36–39]. In addition, CSCs/undifferentiated tumors have the ability to proliferate significantly and give rise to much larger numbers of tumor cells, whereas their differentiated counterparts proliferated less and gave rise to much lower numbers of tumor cells [34]. Consequently, CSCs/undifferentiated tumors form larger tumors and were able to invade and metastasize to other organs, whereas their differentiated counterparts formed smaller tumors and were not able to metastasize [34,40]. Interestingly, although CSCs/undifferentiated tumors were highly susceptible to NK cell mediated cytotoxicity, they were greatly resistant to the effect of chemotherapeutic drugs [32,40].

Therefore, due to significant heterogeneity in tumor cells, it is quite

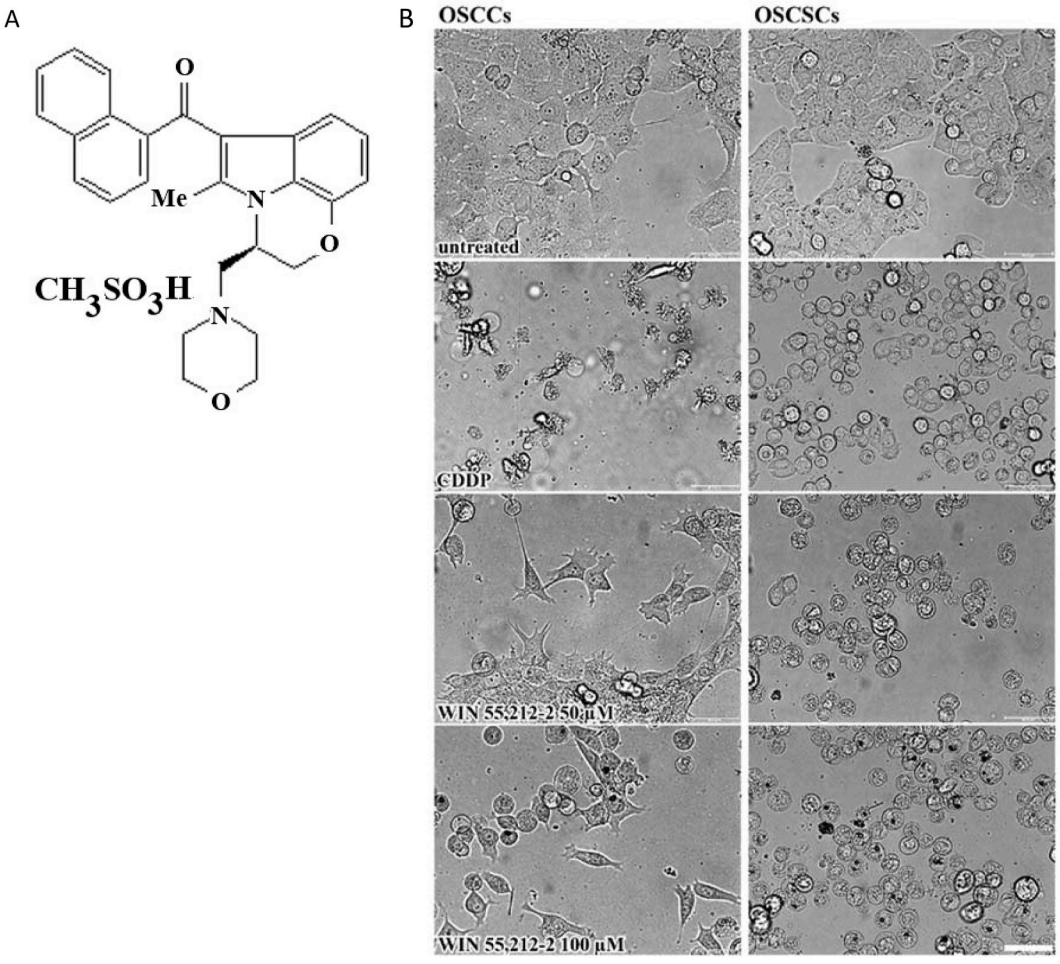

Fig. 1. WIN 55,212–2 treatment induced more pronounced shift in morphology and decreased viability in stem-like OSCSCs more than in differentiated OSCCs. Chemical structure of WIN 55,212–2 (A). Tumor cells were cultured at 3×10^5 cells per ml in 12 well plates and treated with different concentrations of WIN 55,212–2 as shown in the figure, and images of the cells were taken under 400 × magnification using DMI6000 B inverted microscope and LAS X software. OSCCs and OSCSCs were treated with CDDP (50 μg/mL) and different concentrations of WIN 55,212–2 for 24 h before the microscopic images were taken. Scale bar = 50 μm (B).

challenging to eliminate tumors and predict the disease outcome [41]. Despite numerous studies on the anti-tumor effect of WIN 55,212–2, its effect on differentiated vs stem-like tumor cells still remains to be elucidated. In this study, by using the four surface markers of CD44, MHC class I, CD54 and PDL-1we investigated the effects of WIN 55, 212–2 on poorly differentiated/cancer stem like cells (CSCs) and their counterpart, differentiated tumor cells in oral and pancreatic tumors. For the first time, we demonstrate that the anti-proliferative and the direct cytotoxic effect of WIN 55,212–2 are more pronounced on poorly differentiated/CSC-like tumors when compared to their well-differentiated counterparts, even though they can also inhibit the growth and proliferation of well-differentiated tumors. In addition, in our preliminary experiments, by activating NK cells in humanized BLT mice, WIN 55,212–2 was able to increase the cytotoxic function of NK cells against OSCSCs/poorly differentiated tumors.

2. Materials and methods

2.1. Cell lines and reagents

RPMI 1640 (Gibco, Thermo Fisher Scientific, USA) complete medium with 10% fetal bovine serum (FBS) (Gemini Bio-Products, San Diego, CA, USA), 1.4% of non-essential amino acid (Gibco, Thermo Fisher Scientific, USA), 1.4% sodium pyruvate (Gibco, Thermo Fisher Scientific, USA), 0,15% of sodium bicarbonate (Fisher Scientific, Waltham, MA, USA) and 1% antibiotics/antimycotics (Gemini Bio-Products, San Diego, CA, USA) was used for oral tumor culture. DMEM (Gibco, Thermo Fisher Scientific, USA) supplemented with 10% FBS and 1% antibiotics/antimycotics was used for pancreatic tumor cell culture. Oral squamous carcinoma cells (OSCCs) and oral squamous carcinoma stem cells (OSCSCs) were isolated from cancer patients with tongue tumor at UCLA [36–38]. Human pancreatic cancer cell lines MIA PaCa-2 (MP2) and PL-12 were generously provided by Dr. Guido Eibl (UCLA David Geffen School of Medicine) and by Dr. Nicholas Cacalano (UCLA Jonsson Comprehensive Cancer Center), respectively [42]. Antibodies to CD44, MHC class-I, CD54 and PD-L1 used for flow cytometry were purchased from BioLegend (San Diego, CA). Propidium iodide (PI) was purchased from Sigma-Aldrich (St. Louis, MO). WIN 55,212-2 mesylate was purchased from Tocris Bioscience (Bristol, UK). CDDP was purchased from Ronald Reagan UCLA Medical Center Pharmacy. PE conjugated CB1R and Alexa fluor 488 conjugated CB2R antibodies were purchased from Biotechne (NE, MN). TNF-α and IFN-γ were purchased from Peprotech (Rockyhill, NJ).

2.2. Microscopy

Tumor cells were cultured at 3×10^5 cells per ml in 12 well plates and treated with different concentrations of WIN 55,212–2 and CDDP as described in the figure legends and images of the cells were taken under 400× magnification using DMI6000 B inverted microscope and LAS X software (both Leica, Wetzlar, Germany).

2.3. Cell count

Tumor cells were cultured at 3×10^5 cells per ml in 12 well plates and treated with different concentrations of WIN 55,212–2 and CDDP as described in the figure legends, and the detached cells were collected before the wells were washed with 1 × PBS and the attached cells were harvested with trypsin-0.25% EDTA (Gibco, Thermo Fisher Scientific, PA, USA) and they were combined with detached cells and counted. The number of viable cells was counted under light microscope using Trypan Blue staining (Sigma, MO, USA).

2.4. Surface staining and cell death assays

Tumor cells were cultured at 3×10^5 cells per ml in 12 well plates and treated with different concentrations of WIN 55,212–2 and CDDP as described in the figure legends, and the detached cells were collected before the wells were washed with 1 × PBS and the attached cells were harvested with trypsin-0.25% EDTA (Gibco, Thermo Fisher Scientific, PA, USA) and they were combined with detached cells before they were stained with the antibodies and Propidium Iodide (PI) (Sigma, Aldrich). Cell surface receptor staining was performed by labeling the cells with PE-conjugated antibodies against CD44, CD54, PD-L1, CB1R and MHC class I or propidium iodide (PI) and Alexa fluor 488 conjugated CB2R as described previously [43,44]. Attune NxT flow cytometer (Thermo Fisher Scientific, MA, USA) were used to run the samples and the results were analyzed using FlowJo vX software (BD, OR, USA).

2.5. Statistical analysis

A paired or unpaired, two-tailed Student *t*-test was performed for the statistical analysis using Prism-7 software (Graphpad Prism, CA, USA) to compare different groups. The following symbols represent the levels of statistical significance within each analysis, *** (p-value < 0.001), ** (p-value 0.001–0.01), * (p-value 0.01–0.05)

3. Results

3.1. WIN 55,212–2 inhibited cell proliferation and induced cell death in oral tumor cells, and the effect was more pronounced in stem-like OSCSCs

Oral squamous carcinoma cells (OSCCs) and oral squamous carcinoma stem like tumor cells (OSCSCs)/poorly differentiated tumors were treated with different concentrations of WIN 55,212–2 (50–100 μM) and *cis*-diamminedichloridoplatinum (II) (CDDP) (50 μg/mL) for 24 h. CDDP is a chemotherapeutic drug shown to be more cytotoxic to well-differentiated tumor cells previously [32], and was used to compare it's effect to the killing ability of WIN 55,212–2 in this study. No differences in either Forward/Side scatter of the tumor cells representing the health of the tumor cells nor the levels of cell death could be seen between untreated or those treated with the highest concentrations of DMSO vehicle used to solubilize WIN 55,212–2 (Fig. S1A and S1B). When treated with low concentrations of WIN 55,212–2, OSCCs remained viable (data not shown). After treatment with 50 μM or higher concentrations of WIN 55,212–2, some of the OSCCs still remained viable but others rounded up and detached and were not viable (Fig. 1B). OSCSCs, the stem-like tumor cells, became detached from the culture plates when treated with WIN 55,212–2 (Fig. 1B). On the other hand, CDDP caused OSCCs' loss of morphology, and the majority were detached from the plates. Despite the loss of some morphology in OSCSCs, the majority of these cells were still attached to the cell culture plate after CDDP treatment (Fig. 1B).

Cell growth of OSCCs and OSCSCs was evaluated after WIN 55,212–2 (10–100 μM) and CDDP (50 μg/mL) treatments by visually counting the numbers of viable cells using microscopy. WIN 55,212-2 decreased the cell numbers of OSCC by 10–25% after 24 h of treatment, whereas a greater decrease was observed in the cell numbers of OSCSC, ranging from 40% to 65% (Fig. 2). In contrast, CDDP was found to inhibit cell growth of OSCCs more than those seen with OSCSCs (Fig. 2). In addition, we have observed significant dose dependent decrease in forward scatter/side scatter in OSCCs treated with different concentrations of WIN 55,212–2 (Fig. S1B), demonstrating the decrease in the size of these tumors by WIN 55,212–2, a criteria which were previously used to determine the early stages of apoptosis in the cells [45].

Cell death was then evaluated after WIN 55,212–2 and CDDP treatment by using propidium iodide (PI) staining and flow cytometric analysis. Concentrations of 75 and 100 μM WIN 55,212–2 induced cell death in OSCCs up to 10% after 24 h of treatment, however, higher cell death was detected in OSCSCs at all concentrations tested (Fig. 3 and S1C–S1G). As expected, CDDP triggered more cell death in OSCCs when compared to OSCSCs (Fig. S1C, S1E and S1F). Although on average,

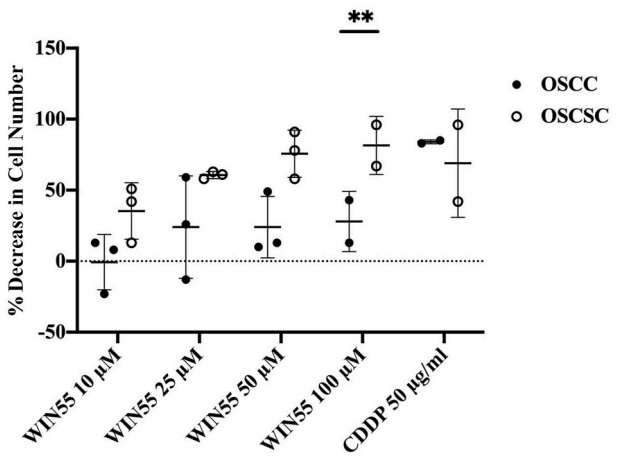

Fig. 2. WIN 55, 212–2 treatment induced a greater decrease in the growth of stem-like/poorly differentiated OSCSCs. Tumor cells were cultured at 3 × 10^5 cells/ml in 12 well plates and treated with different concentrations of WIN 55,212–2 and CDDP as shown in the figure for 24 h, and the detached cells were collected before the wells were washed with 1 × PBS and the attached cells were harvested using trypsin-0.25% EDTA, and they were combined with detached cells and counted. The number of viable cells was counted under light microscope using Trypan Blue staining. Data are shown as Mean ± SD. Compiled data from three independent experiments performed as shown. An unpaired, two tailed Student *t*-test was performed for the statistical analysis using Prism-7 software to compare within treatment group. The following symbol represent the levels of statistical significance within each analysis, ** (p-value 0.001–0.01).

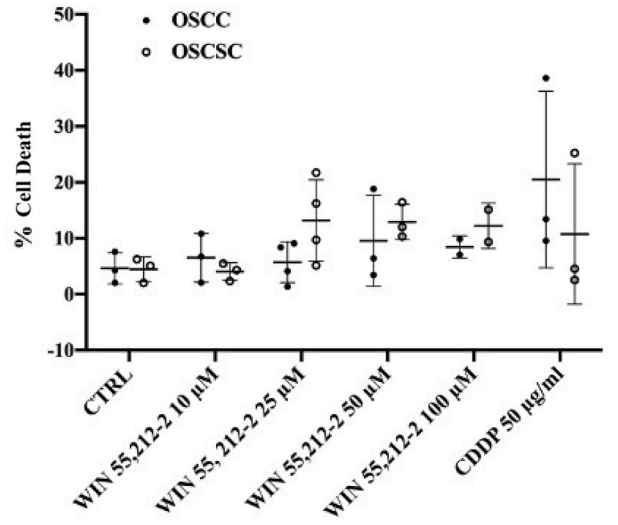

Fig. 3. WIN 55,212–2 induced greater cell death in stem-like OSCSCs when compared to differentiated OSCCs. Tumor cells were cultured at 3 × 10^5 cells per ml in 12 well plates and treated with different concentrations of WIN 55,212–2 and CDDP as shown in the figure for 24 h, and the detached cells were collected before the wells were washed with 1 × PBS and the attached cells were harvested using trypsin-0.25% EDTA, and they were combined with detached cells. PI staining was used to determine the cell death by flow cytometry. Data are shown as Mean ± SD. Compiled data from four independent experiments performed is shown. An unpaired two-tailed Student *t*-test (was performed for the statistical analysis using Prism-7 software.

higher cell death was observed in OSCSCs by WIN 55,212–2 treatment when compared to OSCCs in each single experiment, when considering all the independent experiments between the two different tumors, the values obtained did not achieve statistical significance due to variability between the experiments carried out in different days. The average amount of cell death induced by CDDP was higher in OSCCs when compared to OSCSCs, and the amounts of cell death induced at higher concentrations of WIN 55,212–2 (25–100 μM) in OSCSCs were either similar or higher when compared to those induced by CDDP (Fig. 3 and S1C and S1F). Therefore, WIN 55,212–2 is a potent inducer of cell death in poorly differentiated oral tumor cells.

3.2. WIN 55,212-2 decreased cell surface expression of CD44, CD54, MHC class I and PD-L1 on oral tumor cells

Next, we analyzed cell surface receptor expression of CD44, CD54, MHC class I and PD-L1 on oral tumor cells after different concentrations of WIN 55,212–2 treatment. Decreased expression of CD44, CD54, PD-L1 and MHC class I was detected in OSCCs after WIN 55,212–2 treatment when compared to untreated controls. The effect was most pronounced with the highest concentrations of WIN 55,212–2 (50 μM) (Fig. 4 and S2). Similarly, the expression of CD44, CD54 and PD-L1 in OSCSCs decreased after WIN 55,212–2 treatments. In contrast to OSCCs, MHC class I expression in OSCSCs remained unchanged after WIN 55,212–2 treatment (Fig. 4 and S2). Significantly greater decrease in cell surface MHC class I expression after WIN 55,212–2 treatment was detected in OSCCs when compared to OSCSCs since OSCCs express much higher levels of MHC class I on their surface (Fig. 4).

3.3. Decrease in cell proliferation and induction of cell death by WIN 55,212–2 in stem-like MP2 tumor cells when compared to differentiated PL-12 tumor cells

Well-differentiated PL-12 and poorly differentiated/stem like MP2 pancreatic tumor cells were treated with different concentrations of WIN 55,212–2 for 24 h before their respective cell images were taken by microscopy. The results were compared to the treatment of the tumors with CDDP (Fig. 5). PL-12 tumor cells maintained their morphology and remained viable after WIN 55,212–2 treatment, although some floating and non-viable cells were seen in the culture plates at the highest concentration of WIN 55,212–2 (Fig. 5). In contrast, MP2 tumor cells had largely lost their shape and morphology, and had detached from the plates, and were sickly after treatment with WIN 55,212–2 (Fig. 5). In comparison to WIN 55,212–2, CDDP affected both PL-12 and MP2 tumors morphologically (Fig. 5).

Viable cell numbers in the cell cultures were determined after treatment of tumors with different concentrations of WIN 55,212–2 using microscopy. Decreased numbers of viable cells were seen in both PL-12 and MP2 tumors cells by 50–70% and 60–85%, respectively (Fig. 6). Therefore, there was a significantly greater decrease in cell numbers, in two concentrations, after WIN 55,212–2 treatment in MP2 tumor cells when compared to PL-12 tumors when the independent experiments were compiled from different days. In addition, decreased numbers of MP2 tumor cells by the highest concentrations of WIN 55,212–2 (75 and 100 μM) was similar to those seen in CDDP treated groups (Fig. 6).

Next, we determined the levels of cell death induced by WIN55,212–2 and CDDP using PI staining and flow cytometric analysis. Significantly higher percentages of dead cells were seen in poorly differentiated/stem-like MP2 (approximately 30%) when compared to differentiated PL-12 (up to 20%) after treatment with different concentrations of WIN 55,212–2 (25–100 μM)(Fig. 7 and S3A-S3E). In contrast, cell death induced by CDDP was higher in PL-12 in the representative experiment (Fig. S3A) and similar in compiled data when compared to MP2 tumors due to variability we see among the different experiments (Fig. 7 and S3A-S3D). In the paired compiled experiments, when compared within the same concentration of WIN 55–212,2, PL12 has lower cell death than MP2 tumor cells across treatment groups (Fig. S3D).

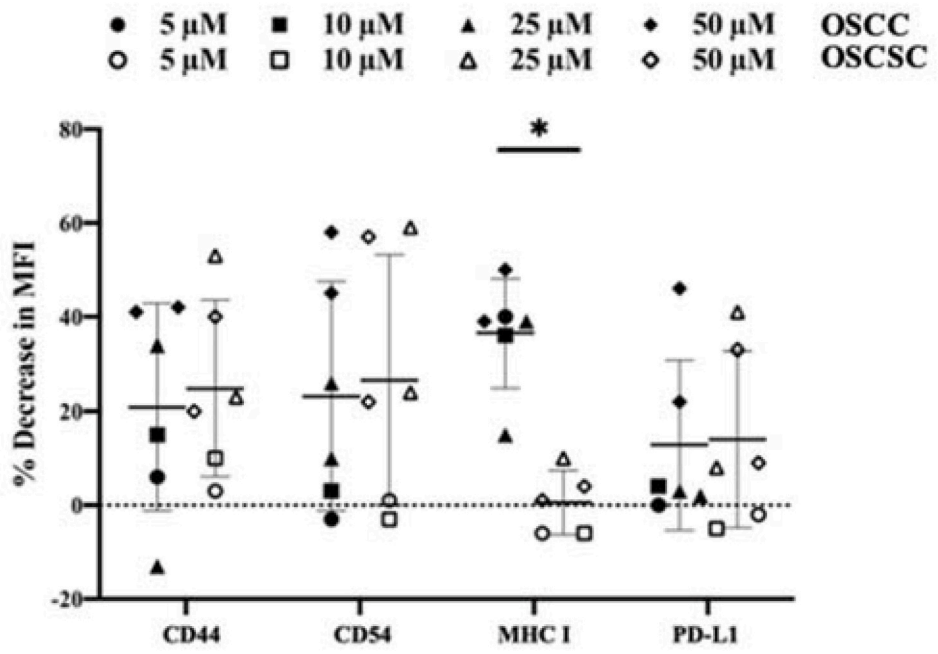

Fig. 4. Significant decrease in MHC class I expression was seen on OSCCs after treatment with different concentrations of WIN 55,212–2. Tumor cells were cultured at 3 × 10^5 cells per ml in 12 well plates and treated with different concentrations of WIN 55,212–2 as shown in the figure, and the detached cells were collected before the wells were washed with 1 × PBS and the attached cells were harvested with trypsin-0.25% EDTA, and they were combined with detached cells before they were stained with the PE conjugated antibodies to CD44, CD54, MHC class-I and PD-L1. Attune NxT flow cytometer were used to assess stained samples and the results were analyzed using FlowJo vX software. Closed symbols represent OSCSCs and open symbols represent OSCCs and a variety of symbols represent different concentrations of WIN 55,212–2 as indicated in the figure (circle: 5 μM, square: 10 μM, triangle: 25 μM, diamond: 50 μM). The results of two independent experiments are shown across a number of different concentrations of WIN 55,212–2. Data are shown as Mean ± SD. An unpaired, two tailed Student *t*-test was performed for the statistical analysis using Prism-7 software to compare within tested cell lines. The following symbol represent the levels of statistical significance within each analysis, * (p-value 0.01–0.05). Mean Flourescence Intensity (MFI).

3.4. Cell surface expressions of CD44, CD54, PD-L1 and MHC class I were down-regulated in pancreatic tumor cells after WIN 55,212–2 treatment

Cell surface expressions of CD44, CD54, PD-L1 and MHC class I were analyzed on PL-12 and MP2 tumor cells after WIN 55,212–2 and CDDP treatments. Decreased cell surface expression of CD44 was seen on both PL-12 and MP2 tumor cells after 48 h of treatment with WIN 55,212–2 (Fig. 8 and S4). Similar to oral tumors, WIN 55,212–2 treatment decreased the expression of CD54 on both MP2 and PL-12 tumor cells. However, the expressions of PD-L1 were seen to be increased on PL-12 but decreased on MP2 tumor cells after WIN 55,212–2 treatment (Fig. 8 and S4). MHC class I expressions were found to be either not changed or slightly decreased on PL-12 tumor cells but decreased on MP2 tumor cells (Fig. 8 and S4)

Taken together, WIN 55,212–2 treatment down-regulated expression of all cell surface receptors in differentiated PL-12 and poorly differentiated/stem-like MP2 tumor cells with the exception of PD-L1, in which expressions were up-regulated on PL-12 tumor cells after treatment. Also, the extent of decrease in all surface receptor expressions on MP2 tumor cells was significantly greater than those seen on PL-12 tumor cells after WIN 55,212–2 treatments (Fig. 8).

3.5. Surface expression of CB2R but not CB1R on oral and pancreatic tumors

To determine whether there is a correlation between the expression of the CB1 and CB2 receptors and higher sensitivity to WIN 55,212–2 effect in tumor cells, we assessed the levels of these receptors on the surface of both stem-like/poorly differentiated and well-differentiated oral and pancreatic tumor cells. We did not observe expression of CB1 receptors on any of the tumor cells tested (Fig. 9A). Surface expressions of CB2 receptors were seen on both oral and pancreatic tumor cells, with well-differentiated tumors having higher expressions than stem-like/poorly differentiated tumor cells (Fig. 9A). As shown in Fig. 9A the levels of differentiation in OSCCs and PL-12 tumor cells is correlated with decreased surface expressions of CD44 when compared to those expressed on the surface of OSCSCs and MP2 tumor cells. We next differentiated the stem-like/poorly differentiated tumor cells with IFN-γ and TNF-α treatment as established in a number of previous publications [36,37,40,42,46], and assessed the levels of receptor expression. Treatment with IFN-γ and TNF-α increased CB2 receptor expression on differentiated stem-like/poorly differentiated tumor cells (Fig. 9B). Therefore, no correlation could be observed for increased CB2 receptor expression and increased cell death in both oral and pancreatic tumor cells. In contrast, we observed an increased CB2 receptor expression on well-differentiated tumor cells with lower induction of cell death after WIN 55, 212–2 treatment in comparison to stem-like/poorly differentiated tumors with lower expression of CB2 receptors but higher induction of cell death after WIN 55, 212–2 treatment.

4. Discussion

Mounting evidence indicates that cannabinoids have profound anti-proliferative and anti-tumor effect against a variety of tumor types. Several studies have also reported the underlying mechanisms of anti-tumor activity of cannabinoids. However, previous studies did not differentiate the effect of cannabinoids on tumor cells in different stages of differentiation. We sought to determine the effect of cannabinoid on both well-differentiated as well as poorly differentiated tumor cells. We and others have previously demonstrated that several CSCs and poorly differentiated tumor cells were resistant to the effects of chemotherapy, whereas their well-differentiated tumor cells were relatively more susceptible [32,37]. Indeed, the primary NK cells activated with IL-2 were the only cell type that was found to target CSCs/poorly differentiated tumor cells but not the well-differentiated tumor cells [47]. Therefore, it is extremely important to find drugs or other factors that can target resistant CSCs/poorly differentiated tumor cells since these tumors seed the cancer and have metastatic potential, unlike the well-differentiated tumor cells [37,47–51]. We report here that synthetic cannabinoid WIN 55,212-2 has the potential to target and kill CSCs/poorly differentiated tumor cells. Although WIN 55,212–2 can also target the well-differentiated tumor cells, its effect is more pronounced on CSCs/poorly differentiated tumor cells. This is different from those seen by chemotherapeutic drugs since these drugs have a greater ability to target well-differentiated tumor cells and in certain tumors they do not

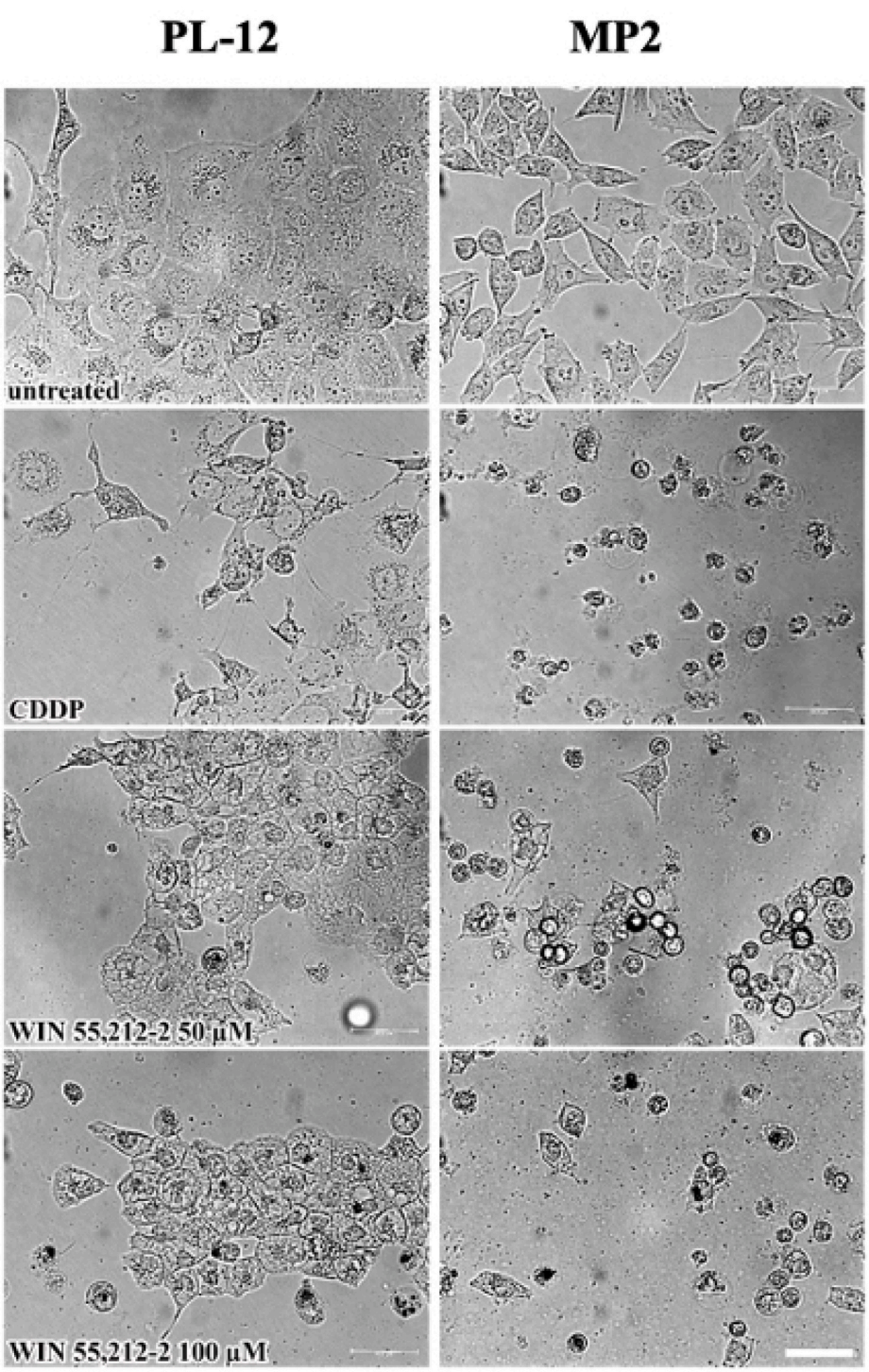

Fig. 5. WIN 55,212–2 treatment induced more pronounced shift in morphology and decreased viability in stem-like MP2 tumor cells more than in well-differentiated PL-12 tumor cells. Tumor cells were cultured at 3×10^5 cells per ml in 12 well plates and treated with different concentrations of WIN 55,212–2 as shown in the figure, and images of the cells were taken under 400 × magnification using DMI6000 B inverted microscope and LAS X software. MP2 and PL-12 tumor cells were treated with CDDP (50 µg/mL) and different concentrations of WIN 55,212–2 for 24 h before the microscopic images were taken. Scale bar = 50 µm.

affect the course of CSCs/poorly differentiated tumor cells [37,47–51].

In our previous studies we have identified and characterized a number of oral and pancreatic tumor lines in different stages of differentiation [36,37,40,42,46]. By using four surface receptors of CD44, CD54, MHC class I and PD-L1, we were able to differentiate between CSCs/poorly differentiated tumor cells, moderately differentiated tumor cells and well-differentiated oral and pancreatic tumor cells [36,37,40,42,46] CSCs/poorly differentiated tumor cells exhibited higher CD44 and lower or no expression of CD54, MHC class I and PD-L1, whereas well-differentiated tumor cells expressed lower CD44 and higher expressions of CD54, MHC class I and PD-L1 [36,37,40,42,46]. These studies formed the basis, and facilitated the work reported in this paper with WIN 55,212-2 since we used these four surface antigens to study the effect of WIN 55,212,2 on oral and pancreatic tumor cells. We observed that tumor cell surface expressions of these four receptors were greatly modulated/decreased on both oral and pancreatic tumors by WIN 55,212–2, likely due to the ability of this compound to block proliferation, induce cell death and/or modulate the surface receptors. The ability to decrease cell surface expression by WIN 55,212–2 were seen on both well-differentiated and CSCs/poorly differentiated tumor cells, even though, WIN 55,212-2 had greater ability to induce decrease in cell numbers and increase cell death in CSCs/poorly differentiated tumor

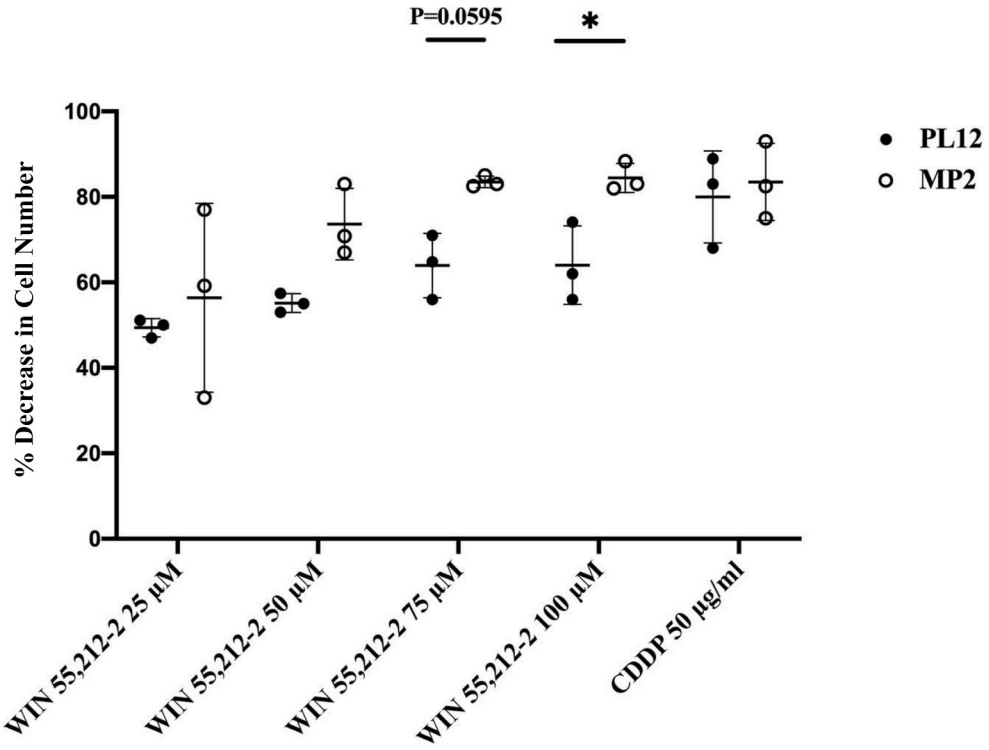

Fig. 6. WIN 55,212–2 caused a greater decrease in cell numbers of stem-like MP2 when compared to PL-12. Tumor cells were cultured at 3×10^5 cells per ml in 12 well plates and treated with different concentrations of WIN 55,212–2 and CDDP as shown in the figure for 24–48 h, and the detached cells were collected before the wells were washed with 1 × PBS and the attached cells were harvested using trypsin-0.25% EDTA, and they were combined with detached cells and counted. The number of viable cells was counted under light microscope using Trypan Blue staining. Data are shown as Mean ± SD. Compiled data from three experiments performed is shown. An unpaired, two tailed Student *t*-test was performed for the statistical analysis using Prism-7 software. The following symbol represent the levels of statistical significance within each analysis, * (p-value 0.01–0.05).

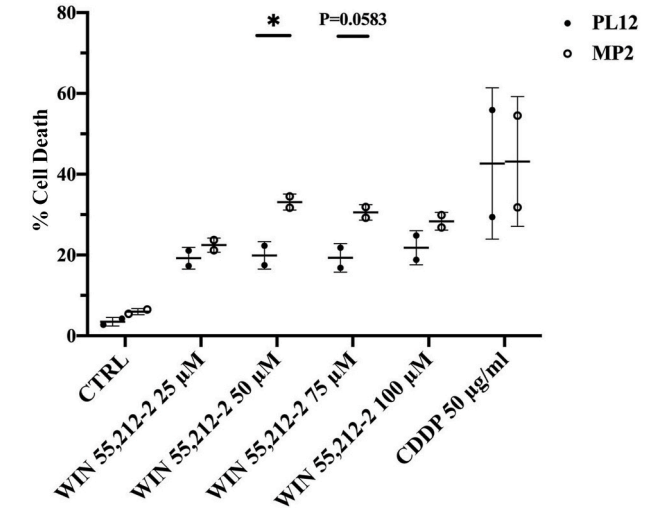

Fig. 7. WIN 55,212–2 induced higher cell death in stem-like MP2 cells compared to differentiated PL-12, whereas the effects of CDDP were pronounced on both PL-12 and MP2. Tumor cells were cultured at 3×10^5 cells per ml in 12 well plates and treated with different concentrations of WIN 55,212–2 and CDDP as shown in the figure for 24–48 h, and the detached cells were collected before the wells were washed with 1 × PBS and the attached cells were harvested using trypsin-0.25% EDTA, and they were combined with detached cells. PI staining was used to determine the cell death by flow cytometry. Data are shown as Mean ± SD. Compiled data from two experiments is shown. An unpaired two-tailed Student *t*-test was performed for the statistical analysis using Prism-7 software. The following symbols represent the levels of statistical significance within each analysis, * (p-value 0.01–0.05), ** (p-value 0.001–0.01).

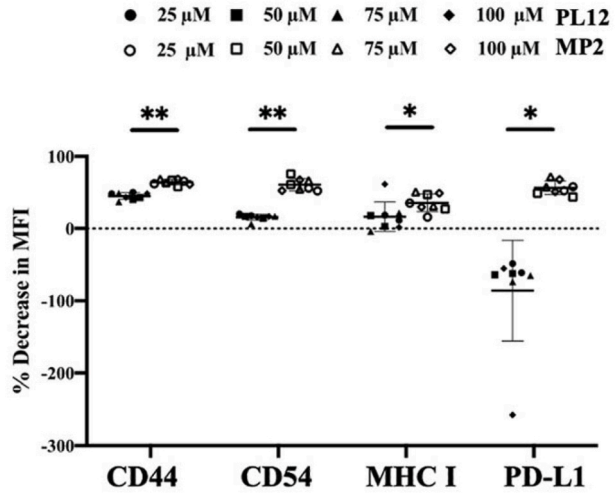

Fig. 8. Surface expressions of CD44, CD54 and MHC class I was decreased on **MP2 and PL-12 tumor cells, while PD-L1 was increased** on **PL-12 and decreased** on **MP2 tumors after WIN 55, 212–2 treatment.** Tumor cells were cultured at 3×10^5 cells per ml in 12 well plates and treated with different concentrations of WIN 55,212–2 as shown in the figure, and the detached cells were collected before the wells were washed with 1 × PBS, and the attached cells were harvested with trypsin-0.25% EDTA, and they were combined with detached cells before they were stained with the PE conjugated antibodies to CD44, CD54, MHC class I and PD-L1. Attune NxT flow cytometer were used to run the samples and the results were analyzed using FlowJo vX software. Closed symbols represent PL-12s and open symbols represent MP2s and a variety of symbols represent different concentrations of WIN 55,212–2 treated as indicated (circle: 25 µM, square: 50 µM, triangle: 75 µM, diamond: 100 µM). The results of two independent experiments are shown across a number of different concentrations of WIN 55,212–2. Data are shown as Mean ± SD. An unpaired, two tailed Student *t*-test was performed for the statistical analysis using Prism-7 software to compare within tested cell lines. The following symbols represent the levels of statistical significance within each analysis, * (p-value 0.01–0.05), ** (p-value 0.001–0.01). Mean Flourescence Intensity (MFI)

cells when compared to well-differentiated tumor cells. This may suggest that perhaps WIN 55,212–2 can also sensitize the well-differentiated tumor cells to NK cell mediated cytotoxicity since it will decrease the levels of MHC class I expression which are known to

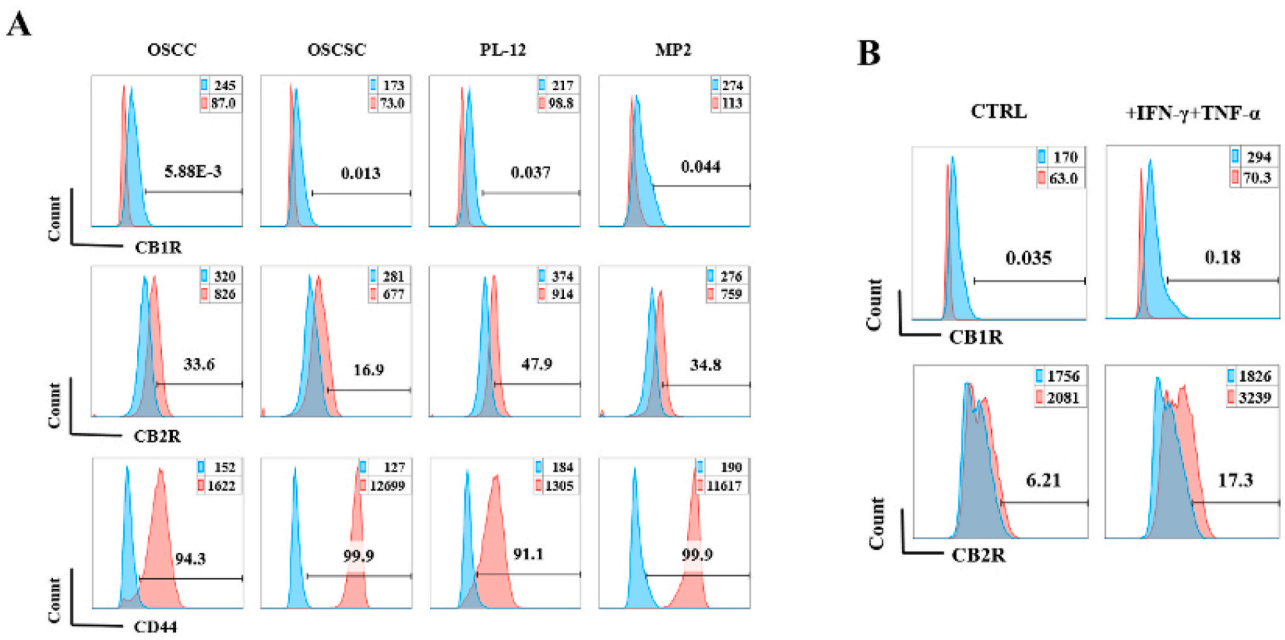

Fig. 9. Surface expressions of CB2R but not CB1R on differentiated and stem-like/poorly differentiated tumor cells with differentiated tumors having higher expression of CB2R. Tumor cells were cultured at 3×10^5 cells per ml in 12 well plates before the wells were washed with 1 × PBS and the tumors were harvested with trypsin-0.25% EDTA before they were stained with the PE conjugated antibodies to CB1R and CD44 and Alexa fluor 488 conjugated CB2R (shown in red). Isotype control antibodies were used to exlcude non-specific staining (shown in blue). Attune NxT flow cytometer were used to run the samples and the results were analyzed using FlowJo vX software. One of two independent experiments is shown in (A). OSCSCs were cultured at 3×10^5 cells per ml in 12 well plates before they were treated with the combination of IFN-γ (10 ng/ml) and TNF-α (10 ng/ml) for 48 h. The tumors were then washed with 1 × PBS and detached by the use of trypsin-0.25% EDTA before they were stained with Alexa fluor 488 conjugated CB2R antbodies. The stained sample were assessed using Attune NxT flow cytometer, and the results were analyzed using FlowJo vX software (B).

inhibit the function of primary activated NK cells. Indeed, in our preliminary experiments in Hu-BLT mice, intraperitoneal injection of WIN 55,212–2 increased the cytotoxic function of NK cells in PBMCs (Fig. S5A and S5B) and augmented IFN-γ secretion (Fig. S5C) and immune-spots in PBMCs (Fig. S5D), and IFN-γ immune-spots in bone marrow (Fig. S5E), spleen (Fig. S5F) and purified $CD3^+$ T cells (Fig. S5G), similar to those observed by the peritoneal injection of IL-15 or feeding with AJ2 probiotic bacteria (Fig. S5). Therefore, not only WIN 55,212–2 can directly target and kill the CSCs/poorly differentiated tumors but also *in vivo* it can also activate the function of NK cells to indirectly target these tumors.

PD-L1 expression is decreased on MP2 tumor cells but not on PL-12 tumor cells, suggesting that WIN 55,212–2 may have differential effects on the expression of PD-L1 on different tumor cell types. PD-L1 is known to inhibit the function of cytotoxic immune effectors by binding to PD-1 [52,53]. Therefore, by decreasing the levels of PD-L1 on stem-like/poorly differentiated tumor cells, WIN 55,212–2 can release the break on the immune cell function and increase their ability to lyse tumors, however, by increasing PD-L1 on PL-12 tumors it may induce the opposite effect, in which it may block the immune function through increased binding to PD-1. It is possible that the decrease in cell surface receptors on tumor cells is due to the ability of WIN 55,212–2 to induce cell death in the tumor cells, but a direct effect of WIN 55,212–2 on surface receptors of tumor cells cannot be ruled out at present. However, it was shown that, cannabinoid use with *anti*-PD-1 agent nivolumab decreased the response rate to therapy in patients with advanced malignancies [54]. Therefore, it is possible that WIN 55,212–2 may have differential effect on tumors depending on the tumor type and their differentiation status.

The effect of WIN 55,212–2 on CSCs/poorly differentiated tumor cells as compared to well-differentiated tumor cells can be due to differential expression of cannabinoid receptors [4]. However, our studies did not demonstrate an increased expression or indicate differential expression of CB1 receptors on either stem-like tumor cells or on their differentiated counterparts (Fig. 9). In contrast, CB2 receptor expressions were higher on differentiated OSCCs and PL-12 tumor cells when compared to stem-like OSCSC and MP2 tumor cells, although stem-like/poorly differentiated tumor cells also exhibited some CB2 expressions (Fig. 9A). In addition, when OSCSCs were differentiated by using the combination of recombinant human IFN-γ and TNF-α in which MHC class I, CD54 and PD-L1 were significantly increased as reported previously [36,37,40,42,46], we could also observe an increase in CB2 receptor expression (Fig. 9B). Studies reported previously, demonstrated expression of CB1 receptors on normal tissues whereas on malignant tissues the levels of CB2 receptors were upregulated, and the expressions were more prominent on the differentiated tumor cells, as reported in our study [55,56]. Indeed, the high expression of CB2 receptors was more frequent in well-differentiated hepatocellular carcinoma (HCC) tissue samples than in normal liver, chronic hepatitis, or less differentiated HCCs [55]. Moreover, similar to those reported in this paper, studies reported by Carracedo et al., demonstrated higher mRNA and protein expression by Western blot analysis of CB2 receptors in Capan2 tumor cells which have a well-differentiated phenotype when compared to MP2 tumor cells [42]. However, in the same study, MP2 tumor cells were found to have significantly higher sensitivity to lower concentrations of THC when compared to Capan2 tumor cells, similar to our study [56]. Thus, contrasting observations on the levels of CB2 receptor expression and ligand function in which an increased CB2 receptor expression was observed in the absence of an increase in the function has been demonstrated in previous studies and in our current study [7,57,58]. Lack of correlation between CB2 receptor expression on OSCSCs versus OSCCs and MP2 versus PL-12 and contrasting functions of WIN 55,212–2 on these tumor cells could potentially be due to differences at the post-receptor signaling pathways in the tumor cells or it can be due to the expression of other receptors yet-to-be identified. These different scenarios are currently under investigation in our laboratory and will be reported in the future studies. In agreement with the higher expression of CB2 receptors on differentiated tumor cells, we also observed

increased induction of CB2 receptor expression when OSCSCs were differentiated by the addition of recombinant human IFN-γ and TNF-α treatment as established in our previous studies [36,37,40,42,46]. In addition, normal colonic tissues expressed CB1 receptors whereas the colonic tumor tissues or colonic epithelium in inflammatory bowel disease were strongly positive for CB2 receptor expression [59,60]. Therefore, at present it is not entirely clear how WIN 55,212–2 functions to kill the oral and pancreatic stem-like tumor cells. However, it is possible, that binding to CB2 receptors on differentiated tumors is sufficient to induce cell death, albeit the levels of cell death are less when compared to those induced in stem-like tumors. Our future studies will focus on delineation of underlying mechanisms which govern the function of WIN55,212–2 in the lysis of stem-like tumors, and its potential role in activation of NK cell function.

5. Conclusion

Overall, our studies indicate that synthetic cannabinoid WIN 55,212-2 has greater killing effect on CSCs/poorly differentiated tumor cells than well-differentiated tumor cells and may have potential to be used in patients with aggressive and metastatic tumors. Whether other cannabinoids behave similarly should await for future investigations.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Patent

UCLA 2020-869 patent entitled “Cannabis Limits Cancer Stem Cell Growth in Poorly Differentiated Cancers”

Author contribution

M.-W.K. prepared study design, performed experiments, data analysis, figure preparation, manuscript writing/editing. B.B. performed experiments and manuscript writing/editing. E.S. performed experiments and manuscript writing/editing. A.J. oversaw funding, and the study design, data analysis and manuscript writing/editing. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by various donors.

Acknowledgments

The authors are grateful for the technical help of Nishant Chovatiya, Paul Wong and Joshua Hughes in this manuscript. We appreciate the help of Dr. Kawaljit Kaur for her insightful comments in editing of the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.adcanc.2022.100043.

References

[1] G. Velasco, C. Sanchez, M. Guzman, Anticancer mechanisms of cannabinoids, Curr. Oncol. 23 (2016) S23–S32, https://doi.org/10.3747/co.23.3080.
[2] A.I. Fraguas-Sanchez, C. Martin-Sabroso, A.I. Torres-Suarez, Insights into the effects of the endocannabinoid system in cancer: a review, Br. J. Pharmacol. 175 (2018) 2566–2580, https://doi.org/10.1111/bph.14331.
[3] Z. Qamri, et al., Synthetic cannabinoid receptor agonists inhibit tumor growth and metastasis of breast cancer, Mol. Cancer Therapeut. 8 (2009) 3117–3129, https://doi.org/10.1158/1535-7163.MCT-09-0448.
[4] A. Preet, et al., Cannabinoid receptors, CB1 and CB2, as novel targets for inhibition of non-small cell lung cancer growth and metastasis, Cancer Prev. Res. 4 (2011) 65–75, https://doi.org/10.1158/1940-6207.CAPR-10-0181.
[5] E. Moreno, M. Cavic, A. Krivokuca, V. Casado, E. Canela, The endocannabinoid system as a target in cancer diseases: are we there yet? Front. Pharmacol. 10 (2019) 339, https://doi.org/10.3389/fphar.2019.00339.
[6] A.G. Silva, et al., WIN55,212-2 induces caspase-independent apoptosis on human glioblastoma cells by regulating HSP70, p53 and Cathepsin D, Toxicol. Vitro 57 (2019) 233–243, https://doi.org/10.1016/j.tiv.2019.02.009.
[7] M.I. Khan, et al., Involvement of the CB2 cannabinoid receptor in cell growth inhibition and G0/G1 cell cycle arrest via the cannabinoid agonist WIN 55,212-2 in renal cell carcinoma, BMC Cancer 18 (2018) 583, https://doi.org/10.1186/s12885-018-4496-1.
[8] D. Xu, J. Wang, Z. Zhou, Z. He, Zhao, Q. Cannabinoid WIN55, 212-2 induces cell cycle arrest and inhibits the proliferation and migration of human BEL7402 hepatocellular carcinoma cells, Mol. Med. Rep. 12 (2015) 7963–7970, https://doi.org/10.3892/mmr.2015.4477.
[9] G. Zhang, H. Bi, J. Gao, X. Lu, Y. Zheng, Inhibition of autophagy and enhancement of endoplasmic reticulum stress increase sensitivity of osteosarcoma Saos-2 cells to cannabinoid receptor agonist WIN55,212-2, Cell Biochem. Funct. 34 (2016) 351–358, https://doi.org/10.1002/cbf.3194.
[10] M.L. Casanova, et al., Inhibition of skin tumor growth and angiogenesis in vivo by activation of cannabinoid receptors, J. Clin. Invest. 111 (2003) 43–50, https://doi.org/10.1172/JCI16116.
[11] S. Sarfaraz, F. Afaq, V.M. Adhami, H. Mukhtar, Cannabinoid receptor as a novel target for the treatment of prostate cancer, Cancer Res. 65 (2005) 1635–1641, https://doi.org/10.1158/0008-5472.CAN-04-3410.
[12] T. Luca, et al., The CB1/CB2 receptor agonist WIN-55,212-2 reduces viability of human Kaposi’s sarcoma cells in vitro, Eur. J. Pharmacol. 616 (2009) 16–21, https://doi.org/10.1016/j.ejphar.2009.06.004.
[13] J. Flygare, K. Gustafsson, E. Kimby, B. Christensson, B. Sander, Cannabinoid receptor ligands mediate growth inhibition and cell death in mantle cell lymphoma, FEBS Lett. 579 (2005) 6885–6889, https://doi.org/10.1016/j.febslet.2005.11.020.
[14] C. Blazquez, et al., Cannabinoid receptors as novel targets for the treatment of melanoma, Faseb. J. 20 (2006) 2633–2635, https://doi.org/10.1096/fj.06-6638fje.
[15] S.M. Emery, et al., Combined antiproliferative effects of the aminoalkylindole WIN55,212-2 and radiation in breast cancer cells, J. Pharmacol. Exp. Therapeut. 348 (2014) 293–302, https://doi.org/10.1124/jpet.113.205120.
[16] K. Greish, et al., Synthetic cannabinoids nano-micelles for the management of triple negative breast cancer, J. Contr. Release 291 (2018) 184–195, https://doi.org/10.1016/j.jconrel.2018.10.030.
[17] S. Xian, N.N. Parayath, H. Nehoff, N.M. Giles, K. Greish, The use of styrene maleic acid nanomicelles encapsulating the synthetic cannabinoid analog WIN55,212-2 for the treatment of cancer, Anticancer Res. 35 (2015) 4707–4712.
[18] M. Todaro, et al., Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4, Cell Stem Cell 1 (2007) 389–402, https://doi.org/10.1016/j.stem.2007.08.001.
[19] L. Ricci-Vitiani, et al., Identification and expansion of human colon-cancer-initiating cells, Nature 445 (2007) 111–115, https://doi.org/10.1038/nature05384.
[20] S. Toden, et al., Oligomeric proanthocyanidins (OPCs) target cancer stem-like cells and suppress tumor organoid formation in colorectal cancer, Sci. Rep. 8 (2018) 3335, https://doi.org/10.1038/s41598-018-21478-8.
[21] J.E. Melsen, G. Lugthart, A.C. Lankester, M.W. Schilham, Human circulating and tissue-resident CD56(bright) natural killer cell populations, Front. Immunol. 7 (2016) 262, https://doi.org/10.3389/fimmu.2016.00262.
[22] Z. Guo, H. Hardin, R.V. Lloyd, Cancer stem-like cells and thyroid cancer, Endocr. Relat. Cancer 21 (2014) T285–T300, https://doi.org/10.1530/erc-14-0002.
[23] M.E. Prince, et al., Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 973–978.
[24] Z. Hu, et al., Targeting tissue factor as a novel therapeutic oncotarget for eradication of cancer stem cells isolated from tumor cell lines, tumor xenografts and patients of breast, lung and ovarian cancer, Oncotarget 8 (2017) 1481–1494, https://doi.org/10.18632/oncotarget.13644.
[25] A. Jewett, et al., Strategies to rescue mesenchymal stem cells (MSCs) and dental pulp stem cells (DPSCs) from NK cell mediated cytotoxicity, PLoS One 5 (2010), e9874, https://doi.org/10.1371/journal.pone.0009874.
[26] H. Ichise, et al., NK cell alloreactivity against KIR-ligand-mismatched HLA-haploidentical tissue derived from HLA haplotype-homozygous iPSCs, Stem Cell Rep. 9 (2017) 853–867, https://doi.org/10.1016/j.stemcr.2017.07.020.
[27] A. Jewett, et al., Natural killer cells preferentially target cancer stem cells; role of monocytes in protection against NK cell mediated lysis of cancer stem cells, Curr. Drug Deliv. 9 (2012) 5–16.
[28] E. Ames, et al., NK cells preferentially target tumor cells with a cancer stem cell phenotype, J. Immunol. 195 (2015) 4010–4019, https://doi.org/10.4049/jimmunol.1500447.
[29] A. Jewett, Y.G. Man, H.C. Tseng, Dual functions of natural killer cells in selection and differentiation of stem cells; role in regulation of inflammation and regeneration of tissues, J. Cancer 4 (2013) 12–24, https://doi.org/10.7150/jca.5519.

[30] B.C. Lee, et al., Adipose natural killer cells regulate adipose tissue macrophages to promote insulin resistance in obesity, Cell Metabol. 23 (2016) 685–698, https://doi.org/10.1016/j.cmet.2016.03.002.
[31] B. Bonamichi, J. Lee, Unusual suspects in the development of obesity-induced inflammation and insulin resistance: NK cells, iNKT cells, and ILCs, Diabetes & metabolism journal 41 (2017) 229–250, https://doi.org/10.4093/dmj.2017.41.4.229.
[32] A.K. Kozlowska, et al., Differentiation by NK cells is a prerequisite for effective targeting of cancer stem cells/poorly differentiated tumors by chemopreventive and chemotherapeutic drugs, J. Cancer 8 (2017) 537–554, https://doi.org/10.7150/jca.15989.
[33] A.K. Kozlowska, et al., Resistance to cytotoxicity and sustained release of interleukin-6 and interleukin-8 in the presence of decreased interferon-gamma after differentiation of glioblastoma by human natural killer cells, Cancer Immunol. Immunother. 65 (2016) 1085–1097, https://doi.org/10.1007/s00262-016-1866-x. CII.
[34] V.T. Bui, et al., Augmented IFN-gamma and TNF-alpha induced by probiotic bacteria in NK cells mediate differentiation of stem-like tumors leading to inhibition of tumor growth and reduction in inflammatory cytokine release; regulation by IL-10, Front. Immunol. 6 (2015) 576, https://doi.org/10.3389/fimmu.2015.00576.
[35] Negro G, Aschenbrenner B, Brezar SK, Cemazar M, Coer A, Gasljevic G, Savic D, Sorokin M, Buzdin A, Callari M, Kvitsaridze I, Jewett A, Vasileva-Slaveva M, Ganswindt U, Skvortsova I, Skvortsov S. Molecular heterogeneity in breast carcinoma cells with increased invasive capacities. Radiol Oncol. 2020 Feb 14;54 (1):103-118. doi: 10.2478/raon-2020-0007. PMID: 32061169; PMCID: PMC7087425. (manuscript in prep).
[36] H.C. Tseng, et al., Increased lysis of stem cells but not their differentiated cells by natural killer cells; de-differentiation or reprogramming activates NK cells, PLoS One 5 (2010), e11590, https://doi.org/10.1371/journal.pone.0011590.
[37] H.C. Tseng, V. Bui, Y.G. Man, N. Cacalano, A. Jewett, Induction of split anergy conditions natural killer cells to promote differentiation of stem cells through cell-cell contact and secreted factors, Front. Immunol. 5 (2014) 269, https://doi.org/10.3389/fimmu.2014.00269.
[38] H.C. Tseng, et al., Differential targeting of stem cells and differentiated glioblastomas by NK cells, J. Cancer 6 (2015) 866–876, https://doi.org/10.7150/jca.11527.
[39] A. Jewett, H.C. Tseng, The Tumor Immunoenvironment, in: Michael Shurin, Viktor Umansky, Anatoli Malyguine (Eds.), Springer, 2013, pp. 361–384.
[40] K. Kaur, et al., Super-charged NK cells inhibit growth and progression of stem-like/poorly differentiated oral tumors in vivo in humanized BLT mice; effect on tumor differentiation and response to chemotherapeutic drugs, OncoImmunology 7 (2018), e1426518, https://doi.org/10.1080/2162402X.2018.1426518.
[41] G.H. Heppner, B.E. Miller, Tumor heterogeneity: biological implications and therapeutic consequences, Cancer Metastasis Rev. 2 (1983) 5–23, https://doi.org/10.1007/BF00046903.
[42] K. Kaur, et al., Probiotic-treated super-charged NK cells efficiently clear poorly differentiated pancreatic tumors in hu-BLT mice, Cancers 12 (2019), https://doi.org/10.3390/cancers12010063.
[43] A. Jewett, B. Bonavida, Target-induced inactivation and cell death by apoptosis in a subset of human NK cells, J. Immunol. 156 (1996) 907–915.
[44] A. Jewett, et al., Cytokine dependent inverse regulation of CD54 (ICAM1) and major histocompatibility complex class I antigens by nuclear factor kappaB in HEp2 tumor cell line: effect on the function of natural killer cells, Hum. Immunol. 64 (2003) 505–520.
[45] A.L. Bertho, M.A. Santiago, S.G. Coutinho, Flow cytometry in the study of cell death, Mem. Inst. Oswaldo Cruz 95 (2000) 429–433, https://doi.org/10.1590/s0074-02762000000300020.
[46] H.C. Tseng, N. Cacalano, A. Jewett, Split anergized Natural Killer cells halt inflammation by inducing stem cell differentiation, resistance to NK cell cytotoxicity and prevention of cytokine and chemokine secretion, Oncotarget (2015).
[47] A. Jewett, et al., Multiple defects of natural killer cells in cancer patients: anarchy, dysregulated systemic immunity, and immunosuppression in metastatic cancer, Crit. Rev. Immunol. 40 (2020) 93–133, https://doi.org/10.1615/CritRevImmunol.2020033391.
[48] K. Kaur, et al., Novel strategy to expand super-charged NK cells with significant potential to lyse and differentiate cancer stem cells: differences in NK expansion and function between healthy and cancer patients, Front. Immunol. 8 (2017) 297, https://doi.org/10.3389/fimmu.2017.00297.
[49] A. Jewett, et al., NK cells shape pancreatic and oral tumor microenvironments; role in inhibition of tumor growth and metastasis, Semin. Cancer Biol. 53 (2018) 178–188, https://doi.org/10.1016/j.semcancer.2018.08.001.
[50] K. Kaur, et al., Natural killer cells target and differentiate cancer stem-like cells/undifferentiated tumors: strategies to optimize their growth and expansion for effective cancer immunotherapy, Curr. Opin. Immunol. 51 (2018) 170–180, https://doi.org/10.1016/j.coi.2018.03.022.
[51] A. Jewett, et al., Natural killer cells: diverse functions in tumor immunity and defects in pre-neoplastic and neoplastic stages of tumorigenesis, Mol Ther Oncolytics 16 (2020) 41–52, https://doi.org/10.1016/j.omto.2019.11.002.
[52] F. Concha-Benavente, et al., PD-L1 mediates dysfunction in activated PD-1(+) NK cells in head and neck cancer patients, Cancer Immunol Res 6 (2018) 1548–1560, https://doi.org/10.1158/2326-6066.CIR-18-0062.
[53] L. Xu, et al., Adipocytes affect castration-resistant prostate cancer cells to develop the resistance to cytotoxic action of NK cells with alterations of PD-L1/NKG2D ligand levels in tumor cells, Prostate 78 (2018) 353–364, https://doi.org/10.1002/pros.23479.
[54] T. Taha, et al., Cannabis impacts tumor response rate to nivolumab in patients with advanced malignancies, Oncol. 24 (2019) 549–554, https://doi.org/10.1634/theoncologist.2018-0383.
[55] X. Xu, et al., Overexpression of cannabinoid receptors CB1 and CB2 correlates with improved prognosis of patients with hepatocellular carcinoma, Cancer Genet. Cytogenet. 171 (2006) 31–38, https://doi.org/10.1016/j.cancergencyto.2006.06.014.
[56] A. Carracedo, et al., Cannabinoids induce apoptosis of pancreatic tumor cells via endoplasmic reticulum stress-related genes, Cancer Res. 66 (2006) 6748–6755, https://doi.org/10.1158/0008-5472.CAN-06-0169.
[57] C.C. Felder, et al., Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors, Mol. Pharmacol. 48 (1995) 443–450.
[58] D. Shire, et al., Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor, Biochim. Biophys. Acta 1307 (1996) 132–136, https://doi.org/10.1016/0167-4781(96)00047-4.
[59] F. Cianchi, et al., Cannabinoid receptor activation induces apoptosis through tumor necrosis factor alpha-mediated ceramide de novo synthesis in colon cancer cells, Clin. Cancer Res. 14 (2008) 7691–7700, https://doi.org/10.1158/1078-0432.CCR-08-0799.
[60] K. Wright, et al., Differential expression of cannabinoid receptors in the human colon: cannabinoids promote epithelial wound healing, Gastroenterology 129 (2005) 437–453, https://doi.org/10.1016/j.gastro.2005.05.026.