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INVITED REVIEW

The Antitumor Activity of Plant-Derived Non-Psychoactive Cannabinoids

Sean D. McAllister[1] · **Liliana Soroceanu**[1] · **Pierre-Yves Desprez**[1]

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Abstract As a therapeutic agent, most people are familiar with the palliative effects of the primary psychoactive constituent of *Cannabis sativa* (CS), Δ^9-tetrahydrocannabinol (THC), a molecule active at both the cannabinoid 1 (CB_1) and cannabinoid 2 (CB_2) receptor subtypes. Through the activation primarily of CB_1 receptors in the central nervous system, THC can reduce nausea, emesis and pain in cancer patients undergoing chemotherapy. During the last decade, however, several studies have now shown that CB_1 and CB_2 receptor agonists can act as direct antitumor agents in a variety of aggressive cancers. In addition to THC, there are many other cannabinoids found in CS, and a majority produces little to no psychoactivity due to the inability to activate cannabinoid receptors. For example, the second most abundant cannabinoid in CS is the non-psychoactive cannabidiol (CBD). Using animal models, CBD has been shown to inhibit the progression of many types of cancer including glioblastoma (GBM), breast, lung, prostate and colon cancer. This review will center on mechanisms by which CBD, and other plant-derived cannabinoids inefficient at activating cannabinoid receptors, inhibit tumor cell viability, invasion, metastasis, angiogenesis, and the stem-like potential of cancer cells. We will also discuss the ability of non-psychoactive cannabinoids to induce autophagy and apoptotic-mediated cancer cell death, and enhance the activity of first-line agents commonly used in cancer treatment.

Keywords Cannabinoid · Cannabidiol · Cancer · Reactive oxygen species

Endocannabinoids

The endocannabinoid system was discovered through research focusing on the primary psychoactive active component of *Cannabis sativa* (CS), Δ^9-tetrahydrocannabinol (THC), and other synthetic cannabinoids (Pertwee 1997). The discovery that THC activated two G protein-couple receptors (GPCRs) termed cannabinoid 1 (CB_1) and cannabinoid 2 (CB_2) prompted the search for the endogenous cannabinoid ligands (Mechoulam et al. 1995; Sugiura et al. 1995). To date multiple putative ligands termed endocannabinoids have been isolated, all consisting of arachidonic acid linked to a polar head group (Piomelli 2003). Within the body, endocannabinoids interact with cannabinoid receptors, and are synthesized, removed, and degraded through specific pathways that are still being defined (Pertwee 2006). The endocannabinoid system has been shown to modulate a wide array of physiological process including learning and memory, appetite, pain, and inflammation (Wilson and Nicoll 2002; Klein 2005).

Plant-Derived Cannabinoids

While there are more than 60 cannabinoids in CS, those present in reasonable quantities include THC, cannabidiol (CBD), cannabinol (CBN), cannabichromene (CBC), and cannabigerol (CBG) (McPartland and Russo 2001). Other major classes of compounds in marijuana include nitrogenous compounds, sugars, terpenoids, fatty acids, and flavonoids (Turner et al. 1980; Albanese et al. 1995; McPartland and

✉ Sean D. McAllister
mcallis@cpmcri.org

[1] California Pacific Medical Center Research Institute, 475 Brannan Street, Suite 220, San Francisco, CA 94107, USA

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Russo 2001). McPartland and Russo reported that the concentration range of THC in the dry weight of marijuana was 0.1–25 %, 0.1–2.9 % for CBD, 0–1.6 % for CBN, 0–0.65 % and 0.03–1.15 % for CBG. Of these, CBD has been studied the most extensively (Zuardi 2008). CBD has been reported to be devoid of psychoactive effects (Hollister and Gillespie 1975), is an anti-arthritic agent (Malfait et al. 2000), anxiolytic (Guimaraes et al. 1994), anti-convulsant (Turkanis and Karler 1975), a neuroprotective agent (Hampson et al. 1998), and inhibits cytokine production (Srivastava et al. 1998), to name a few characteristics. There are reports that it interferes with THC metabolism in vivo (Bornheim and Grillo 1998) and in vitro (Jaeger et al. 1996). CBD easily penetrates the brain (Alozie et al. 1980), but has low binding affinity for cannabinoids receptors and has been shown to act as cannabinoids receptor antagonist in certain models (Huffman et al. 1996; Pertwee 1997). CBD has been reported to have either no effect, enhance, or antagonize the effects of THC in laboratory animals (Brady and Balster 1980; Hiltunen et al. 1988) and in humans (Hollister and Gillespie 1975; Dalton et al. 1976; Hunt et al. 1981).

CBN is another marijuana constituent that has attracted considerable attention. It has weak THC-like effects (Perez-Reyes et al. 1973; Hiltunen et al. 1988; Hiltunen et al. 1989) and weak affinity for the cloned cannabinoid receptors (Huffman et al. 1996). After oral administration, CBN (40 mg/kg) had little influence on THC (20 mg/kg) pharmacokinetics in humans (Agurell et al. 1981). CBD has been reported to attenuate CBN's effects (Jarbe and Hiltunen 1987) in one study and no effect in another (Hiltunen et al. 1988). As for the other constituents in marijuana, little is known about their pharmacological and toxicological properties. CBC is not pharmacologically active in monkeys (Edery et al. 1971), lacks anticonvulsant effects (Karler and Turkanis 1979), but was reported to produce CNS depression and slight analgesia in rodents (Davis and Hatoum 1983). CBG does not stimulate adenylyl cyclase (Howlett 1987), but does appear to have some weak analgesic properties (McPartland and Russo 2001).

Overview of CB_1 and CB_2 Receptor Agonists as Antitumor Agents

In cancer cell lines, CB_1 and CB_2 agonists were first shown to modulate the activity of ERK, p38 MAPK and JNK1/2 (Galve-Roperh et al. 2000; McKallip et al. 2006; Sarfaraz et al. 2006; Ramer and Hinz 2008). However, there was a clear difference in the activity produced (sustained stimulation vs. inhibition) depending upon the agonist used and the cancer cell line studied. Initially, production of ceramide leading to sustained up-regulation of ERK activity by treatment with CB_1 and CB_2 agonists was shown to be an essential component of receptor-mediated signal transduction leading to the inhibition of brain cancer cell growth both in culture and in vivo (Galve-Roperh et al. 2000; Velasco et al. 2004). In targeting primary tumor growth, this was later refined to include de novo-synthesis of ceramide leading to endoplasmic reticulum (ER) stress and induction of TRIB3 resulting in inhibition of pAkt/mTOR and the production of autophagy-mediated cell death (Carracedo et al. 2006a; Carracedo et al. 2006b; Salazar et al. 2009). Multiple reviews focusing on the antitumor activity of CB_1 and CB_2 receptor agonists have been published (Bifulco and Di Marzo 2002; Flygare and Sander 2008; Sarfaraz et al. 2008; Freimuth et al. 2010; Velasco et al. 2012).

Non-Psychoactive Plant-Derived Cannabinoids as Inhibitors of Cancer

With the discovery that CB_1 and CB_2 agonists demonstrated antitumor activity, several groups began to investigate the potential antitumor activity of additional plant-derived cannabinoids (Table 1). These compounds either do not activate or are inefficient at activating CB_1 receptors, which means they produce little to no psychoactivity. Of these compounds, CBD has been the most extensively studied. As opposed to THC, the pathways responsible for antitumor activity of CBD, particularly in vivo, are just beginning to be defined. In culture, the most unifying theme for CBD-dependent inhibition of cancer cell aggressiveness is the production of reactive oxygen species (ROS) (Ligresti et al. 2006; Massi et al. 2006; McKallip et al. 2006; McAllister et al. 2011; Shrivastava et al. 2011; Massi et al. 2012; De Petrocellis et al. 2013). Recently, Singer et al. provided the first evidence in vivo that CBD-dependent generation of ROS is in part responsible for the antitumor activity of the cannabinoid. In tumors derived from glioma stem cells (GSCs), CBD inhibited disease progression, however, a portion of therapeutic resistance to the treatment in this subpopulation of tumor cells was the upregulation of anti-oxidant response genes (Singer et al. 2015).

Paradoxically, CBD is neuroprotective and multiple groups have shown selectivity for CBD inhibition of cancer cell growth in comparison to matched non-transformed cells (Massi et al. 2006; Shrivastava et al. 2011). Additionally, CBD has been shown to have antioxidant properties in neuronal cultures (Hampson et al. 1998). As discussed below, even with the lack of interaction with classical cannabinoid receptors, non-psychoactive cannabinoids appear to share similar mechanisms of action for targeting tumor progression.

Inhibition of Cancer Cell Survival and Tumor Progression

Massi et al. first reported that CBD could inhibit human GBM viability in culture and that the effect was reversed in the

Table 1 Overview of cannabinoids and their effects on aggressive cancers

Cancer	Compound	Phenotype	Major mechanism	Antagonism	Reference
Rat glioma	CBD	↓ cell viability	n.d.	n.d.	Jacobsson et al. 2000
Human glioblastoma	CBD	↓ cell viability ↑ apoptosis ↓ tumor growth	↑ ROS (implied by rescue experiments with TOC)	CB2: partial TOC: full Ceramide inhibitors: none PTX: none	Massi et al. 2004
Multiple human cancers	CBD-hydroxyquinone (HU-331)	↓ cell viability ↓ tumor growth (colon cancer)	n.d.	n.d.	Kogan et al. 2004
Human glioblastoma	CBD	↓ cell migration	n.d.	CB1: none CB2: none PTX: none CPZ: none	Vaccani et al. 2005
Human glioblastoma	CBD	↓ cell viability ↑ apoptosis	↑ caspases ↑ cytochrome C ↑ ROS ↓ GSH	n.d.	Massi et al. 2006
Human leukemia	CBD	↓ cell viability ↑ apoptosis ↓ tumor growth and ↑ apoptosis in vivo	↓ procaspases and ↑ caspases ↓ PARP ↓ Bid ↑ cytochrome C ↑ ROS ↑ Nox4 and $p22^{phox}$ ↓ p-p38 MAPK	CB_1: none CB_2 : partial CPZ: none TOC: partial to full Apocynin: partial to full DPI: partial	McKallip et al. 2006
Multiple human cancers	Plant-derived and purified CBD CBD-A CBG CBC THC THC-A	↓ cell viability ↑ apoptosis in some cells ↓ tumor growth ↓ metastasis	↓ G1/S cell cycle arrest in some cells	CB_2 : partial TRPV1: partial	Ligresti et al. 2006
Human leukemia	CBD	↑ P-glycoprotein substrate accumulation ↑ cytotoxic effects of vinblastine	↓ P-glycoprotein expression	n.d.	Holland et al. 2006
Human lines expressing P-glycoprotein	CBD, CBN, THC, THC-COOH	↑ P-glycoprotein substrate accumulation ↑ cytotoxic effects of ABCG2 substrates e.g., topotecan	n.d.	n.d.	Zhu et al. 2006
Multiple human cancer cell lines	CBD-hydroxyquinone (HU-331)	↓ cell viability	↓ topoisomerase II activity -not dependent on production of ROS	CB_1: none CB_2: none	Kogan et al. 2007
Mouse cell line over-expressing ABCG2 vs. wild-type	CBN, CBD, THC	↑ P-glycoprotein substrate accumulation ↓ ABCG2 ATPase activity ↑ cytotoxic effects of ABCG2 substrates e.g. topotecan		No CB_1, CB_2, or TRPV1 receptors detected by rtPCR	Holland et al. 2007
Human glioblastoma	CBD	↓ tumor growth	↓ 5-LOX ↑ leukotriene B4 ↓ AEA	n.d.	Massi et al. 2008

Table 1 (continued)

Cancer	Compound	Phenotype	Major mechanism	Antagonism	Reference
			↑ FAAH activity		
Human cervical and lung cancer	CBD	↓ invasion ↓ metastasis	↓ TIMP1	CB_1, CB_2, VR_1: full	Ramer et al. 2010a
Human lung cancer	CBD	↓ invasion ↓ tumor growth	↓ PAI	CB_1, CB_2, VR_1: partial	Ramer et al. 2010b
Rat oligodendrocytes	CBD	↓ cell viability	↑ IC calcium ↑ ROS	CB_1: none CB_2: none A_{2A} PPARγ: none	Mato et al. 2010
Human glioblastoma	CBD+THC	↓ cell viability ↓ invasion ↑ apoptosis	↓ Id1 ↑ caspase 3, 7, 9 ↑ PARP ↑ ROS	THC+CBD-CB_2:partial CBD- CB_2: none	Marcu et al. 2010
Human and mouse breast cancer	CBD	↓ invasion ↓ metastasis	↑ ROS ↓ Id1 ↓ G1/S transition ↑ pERK	TOC: partial ERK: partial	McAllister et al. 2010
Human glioblastoma	Plant-derived and purified CBD THC CBD+THC TMZ TMZ+CBD+THC	↓ cell viability ↓ tumor growth •CBD enhanced the antitumor activity of THC •THC or THC+CBD enhanced the antitumor activity of TMZ	↑ autophagy ↑ caspase 3 ↑ tunnel	CBD CB_1: none CB_2: none siATG1:none CBD±THC CB_1: partial CB_2: partial siATG1:partial ISP: partial 3-MA: partial QVDOPH: full TOC: partial NAC: partial	Torres et al. 2011
Human breast cancer	CBD	↓ cell viability ↑ apoptosis	↑ ROS ↑ autophagy ↓ pAKT ↑ PARP ↓ mTOR ↓ 4EBP1 ↑ caspases ↑ t-Bid ↑ Bax * (see paper for additional pathways)	Blocked by TOC and caspase inhibitor. Not blocked by CB_1, CB_2, A_{2A} or PPARγ receptor antagonists.	Shrivastava et al. 2011
Human endothelial cells	CBD	↓ migration ↓ angiogenesis	↓ MMP2, 9 ↓ uPA ↓ Endothelin-1 ↓ PGDF-AA ↓ CXCL16 ↓ PAI-1	n.d.	Solinas et al. 2012

Table 1 (continued)

Cancer	Compound	Phenotype	Major mechanism	Antagonism	Reference
			↓ IL-8		
Human prostate cancer	CBD+several additional plant-derived and purified cannabinoids	↓ cell viability ↓ tumor growth •CBD enhanced or inhibited the antitumor activity of first-line agents depending on the cell line used	↑ calcium ↑ ROS ↑ caspases ↑ p53 ↑ tunel ↓ G1/S transition ↑ p21 ↑ PUMA ↑ CHOP	TRPM8: partial	De Petrocellis et al. 2013
Human lung cancer	CBD	↓ cell viability ↓ tumor growth	↑ COX-2 ↑ PPARγ ↑ PGE2 ↑ PGD2 ↑15d-PGJ2	COX-2: full PPARγ: full	Ramer et al. 2013
Human glioblastoma	CBD	↓ invasion ↓ neurosphere formation ↓ tumor growth	↓ Id1 ↓ Sox2 ↓ pAKT ↓ mTOR ↓ pERK ↓ Beta-catenin ↓ PLCG1	n.d.	Soroceanu et al. 2013
Human breast cancer	CBD and additional cannabinoids	CBD and THC did not interfere with the effectiveness of radiation		n.d.	Emery et al. 2014
Human and mouse breast cancer	CBD	↓ cell viability ↑ apoptosis ↓ advanced stage metastasis ↑ survival	↑ ROS ↓ Id1 ↑ autophagy	CB_1: none CB_2: none TOC: partial to full	Murase et al. 2014
Human and rat glioblastoma	Plant-derived and purified CBD, THC, CBD+THC	↓ cell viability ↓ tumor growth ↑ apoptosis ↓ angiogenesis •CBD+THC enhanced the effects of radiation	↑ pEPK ↑ γ-H2AX ↓ pAKT ↑ autophagy ↑ caspase 3	n.d.	Scott et al. 2014
Human glioblastoma	CBD	↓ cell viability ↓ tumor growth ↓ invasion ↓ GSC self-renewal ↑ ROS	↓ Id1 ↓ pSTAT3 ↓p-p38MAPK ↓pAKT ↓Olig2 ↓Sox2 ↑ CD44 ↑ NRF2 ↑ SLC7A11	n.d.	Singer et al. 2015
Human breast cancer	CBD	↓ cell proliferation ↓ colony formation ↓ migration	↓ Nf-kB ↓ pEGFR ↓ pAKT	n.d.	Elbaz et al. 2015

Table 1 (continued)

Cancer	Compound	Phenotype	Major mechanism	Antagonism	Reference
		↓ invasion ↓ primary tumor growth ↓ metastasis ↓ angiogenesis ↓ macrophage recruitment to tumor	↓pERK ↓MMP2 ↓MMP9 ↓GM-CSF ↓CCL3 ↓CXCL2 ↓arginase-I ↓F4/80 ↓CD11b		
Human melanoma	CBD+THC	↓ cell viability ↓ tumor growth	↑ autophagy ↑ apoptosis	n.d.	Armstrong et al. 2015

↓, down-regulated; ↑, up-regulated; *n.d.* not determined

presence of ROS scavenger a-tocoherol/vitamin E (Massi et al. 2004). In this study, CBD was also shown to inhibit tumor progression in a model where the GBM cells were implanted subcutaneously in xenograft mouse model. An early report by Jacobsson et al. demonstrated CBD could inhibit the viability of a rat glioma (C6) in culture, but no mechanism of action was reported (Jacobsson et al. 2000). Of note, this group demonstrated that the ability of plant-derived cannabinoids to inhibit cancer cell viability was enhanced under culturing conditions where the cells were serum-starved. This phenomenon appears to be primarily the result of the plant-derived cannabinoids binding to high molecular weight serum proteins (De Petrocellis et al. 2013).

Massi et al. went on to demonstrate that CBD-dependent production of ROS was accompanied by reduction in glutathione (GSH) and GSH-related enzymes (Massi et al. 2006). GSH is an important antioxidant that prevents damage to cellular components by ROS. The source of CBD-dependent stress in part originated in the mitochondria and led to activation of multiple caspases involved in intrinsic and extrinsic pathways of apoptosis. Further studies analyzing CBD-treated GBM tumor tissue revealed that inhibition of lipoxygenase (LOX) signaling played a role in CBD antitumor activity. In addition, the indirect modulation of the endocannabinoid system by CBD may have attributed to the observed antitumor activity. It should be noted that a CBD-hydroxyguinone (HU-311) was shown to inhibit prostate cancer cell viability and tumor progression in vivo (Kogan et al. 2004). It was later demonstrated that HU-311 inhibited topoisomerase II (Kogan et al. 2007) and HU-311 did not increase the production of ROS. A detail study of CBD-induced apoptosis was performed in human leukemia cells (McKallip et al. 2006). The investigators demonstrated a CBD-dependent increase in ROS production as well as an increase in the expression of the NAD(P)H oxidases Nox4 and $p22^{phox}$. CBD treatment perturbed the function of the mitochondria as suggested by loss of mitochondrial membrane potential and release of cytochrome c. The cumulative cellular stress led to activation of multiple intrinsic and extrinsic caspases. Importantly, CBD treatment inhibited tumor progression and induced apoptosis in vivo.

Interaction of CBD With Specific Receptors in Models of Cancer

A majority of investigations demonstrate that the ability of non-psychoactive cannabinoids (primarily CBD) to inhibit cancer cell viability/proliferation is not linked to direct interactions with CB_1 and CB_2 receptors, transient receptor potential cation channel subfamily V member 1 (TRPV1), adenosine A2A receptor (A_{2A}) or the peroxisome proliferator-activated receptor gamma (PPRγ) (Table 1). In certain cancer cell lines, the ability of CBD to inhibit cancer cell viability/

proliferation has been reversed in the presence of antagonists for CB_2, TRPV1, TRPM8, COX-2, and PPRγ (Table 1). Lung cancer cell lines appear to be particularly responsive to reversal of the anti-invasive effects of CBD in culture with antagonists to CB_1, CB_2, and TRPV1 (Ramer et al. 2010a; Ramer et al. 2010b). Currently, only two studies have investigated receptor dependence of CBD-dependent antitumor activity in vivo. In a model where tumors were derived from subcutaneous implanted human lung cancer cells, full reversal of CBD-dependent antitumor activity was observed in the presence of a PPRγ antagonist (Ramer et al. 2013). It was recently demonstrated that the anti-metastatic activity of CBD in a mouse model of breast cancer was not reversed in the presence of a CB_2 receptor antagonist (Murase et al. 2014).

CBD-Dependent Release of Calcium from Intracellular Stores

The initial events leading to CBD-dependent production of ROS in cancer cells is still not well understood. In hippocampal cultures, CBD induces a mitochondrial-dependent release of calcium. Under physiological conditions, CBD caused a subtle rise in calcium, but under high-excitability conditions CBD prevented calcium oscillations leading to neuroprotection (Ryan et al. 2009). In oligodendrocytes, however, CBD produced a concentration-dependent increase in calcium leading to alterations in mitochondrial membrane potential, production of ROS, and ultimately cytotoxicity Mato et al. (2010). In these cultures, the activity of CBD was not related to the activation of CB_1, CB_2, or TRPV1. Ligresti et al. also demonstrated that the ability of CBD to inhibit breast cancer cell viability through generation of ROS was calcium-dependent (Ligresti et al. 2006). Recently, it was demonstrated that CBD induced cell death in immortalized BV-2 microglial cells in part through inhibition of voltage-dependent anion channel 1 located in the mitochondrial outer membrane (Rimmerman et al. 2013). The CBD-dependent inhibition of this channel led to a biphasic increase in intracellular calcium levels leading to changes in mitochondrial function and morphology, and production of ROS. Taken together, these studies suggest that CBD interacts with unique mitochondrial sites leading to modulation of calcium homeostasis and ROS.

ROS can exert different effects according to the basal metabolic rate of the cell, and the high basal metabolic rate of cancer cells makes them more susceptible to redox-directed therapeutics in comparison to non-transformed cells (Laurent et al. 2005). The ability of CBD to more selectively reduce the viability/proliferation of cancer cells in comparison to non-transformed cells may be related to differences in metabolic rate, particularly considering that the origin of CBD-dependent production of ROS appears to stem primarily from the mitochondria. Redox-directed therapeutics have been developed to act as direct inhibitors of cancer and to sensitize tumors to first-line agents; however, they are associated with significant toxicity (Wondrak 2009). Therefore, the discovery of natural non-toxic plant-based molecules that selectively up-regulate ROS in tumor cells would be beneficial.

A Diverse Range of Cannabinoids can Induce ER Stress and the Production of ROS

Separate groups performed an unbiased screen of multiple plant and synthetic cannabinoids, and determined that CBD was consistently more potent at reducing cell viability/proliferation in comparison to classical CB_1 and CB_2 receptor agonists, including THC. While this holds true in culture, only two direct comparison of the antitumor activity of a classical CB_1 and CB_2 receptor agonist (THC) and CBD have been carried out in vivo. In tumors derived from subcutaneous implanted human GBM, THC but not CBD could produce moderate inhibition of tumor progression. However, in this study, it was demonstrated that the addition of CBD to THC enhanced its effects (Torres et al. 2011) thereby allowing a lower dose of THC to be used to produce equivalent antitumor activity. This study was in agreement with studies in culture (Marcu et al. 2010) demonstrating that CBD enhanced the inhibitory effects of THC on human GBM cell proliferation and survival. The ability of CBD to enhance the activity of THC was associated with the production of ROS leading to inhibition of pERK and activation of multiple caspases. In a more recent study, it was reported in a mouse model of breast cancer metastasis that CBD was more potent than THC at inhibiting the formation of lung metastatic foci (McAllister et al. 2007; Murase et al. 2014).

Another study in culture by Shrivastava et al. showed that targeting the human breast cancer cell line, MDA-MB231, with CBD led to endoplasmic reticulum stress, inhibition of the AKT/mTOR pathway, and up-regulation of autophagy-mediated cell death (Shrivastava et al. 2011). As discussed earlier, the targeting of these key pathways is also linked to the antitumor activity of CB_1 and CB_2 receptor agonists such as THC (Carracedo et al. 2006b; Salazar et al. 2009; Guindon and Hohmann 2011). Shrivastava et al. provided evidence that the effects of CBD were not the result of interactions with CB_1 and CB_2 receptors (Shrivastava et al. 2011). An intriguing recent study demonstrated that structurally diverse cannabinoids, including a CB_1 selective agonist, a CB_1 selective antagonist, and an endogenous CB_1 and CB_2 mixed agonist could all stimulate the generation ROS and production of autophagy in pancreatic tumor cells (Donadelli et al. 2011). This group went on to further demonstrate that CB_1-selective and CB_2-selective receptor agonists induced autophagic cell death in part through the initial induction of the ROS sensor AMP-activated protein kinase (AMPK) (Dando et al. 2013). More recently, CBD has been shown to enhance the ability of THC to inhibit tumor progression in mice bearing BRAF wildtype

melanoma xenografts through activation of autophagy-mediated cell death (Armstrong et al. 2015).

Of interest is an investigation by Sarker and Maruyama, where this group demonstrated that the endocannabinoid anadamide induced cell death in multiple cancer cell lines (Sarker and Maruyama 2003). In this study, it was demonstrated that formation of lipid rafts, leading to production of ROS, where part of the proposed mechanisms for the observed effects. Taken together, these data suggest that a diverse range of cannabinoids can induce ER stress and ROS generation, regardless of whether they activate or inhibit cannabinoid receptors, or in the case of CBD, do not efficiently target either CB_1 or CB_2 receptors. An important caveat in these studies is that modulation of autophagy and other proposed mechanisms were not confirmed in vivo. This is important since in GBM cells, while both THC and CBD were effective at reducing cell viability/proliferation in culture, only THC was effective at directly inducing autophagy in vivo (Torres et al. 2011). CBD however was able to enhance the ability of THC to induce autophagy, similar to what was reported in a mouse model of melanoma (Armstrong et al. 2015). In another recent study, where multiple GSCs were treated with CBD and then analyzed using Affymetrix microarrays, a robust up-regulation of TRIB3 was observed in all lines (Singer et al. 2015). In addition, in GSC-derived intracranial tumors treated with CBD, a marked down-regulation of pAKT activity was observed. Upregulation of TRIB3 and inhibition of pAKT are hallmarks of autophagy-mediated cell death (Cardaci et al. 2012; Sui et al. 2013). It has been shown that CBD is less potent than THC at inducing autophagy in human breast cancer cells (Murase et al. 2014). The concentration used in culture and doses used in vivo in the Torres et al. study where significantly lower than those used in the studies where up-regulation of autophagy was observed. This may explain the discrepancy between studies.

Inhibition of Invasion and Metastasis

An exciting recent area of investigation for the therapeutic application of CBD resides in its ability to inhibit invasion and metastasis (Ligresti et al. 2006; McAllister et al. 2007; McAllister et al. 2010; Ramer et al. 2010a). While several cancer therapeutics on the market have been designed to target tumor cell survival, none have been specifically designed to inhibit metastasis. Migration is an important step in the process of metastasis. Vaccani et al. first reported that CBD could inhibit glioma cell migration (Vaccani et al. 2005). This effect could not be blocked by a CB_1 or CB_2 receptor antagonist or by pertussis toxin, an inhibitor of Gi subunits of G-proteins. This however does not rule out the possibility that the effects of CBD on cell migration are produced through GPCR receptors signaling utilizing pertussis toxin insensitive G proteins such as Gq or $G_{12}/_{13}$ (Baldwin 1994).

Local invasion of cancer cells followed by invasion to secondary sites is one of the major hallmarks of metastasis. Therefore, in addition to inhibit of cancer cell migration, several groups have demonstrated that CBD could inhibit the invasion and metastasis of aggressive cancer cells (Ligresti et al. 2006; McAllister et al. 2007; Ramer et al. 2010a; McAllister et al. 2011; Ramer et al. 2011; Ramer et al. 2012; Soroceanu et al. 2013; Murase et al. 2014). Particularly, CBD turned off the expression of an important pro-metastatic gene, Id1, in breast and brain cancer cells in culture and in animal models (McAllister et al. 2007; Soroceanu et al. 2013; Murase et al. 2014). Id1 has been shown to play a key role in mediating breast cancer progression and metastasis to the lung (Fong et al. 2003; Minn et al. 2005; Gupta et al. 2007; Swarbrick et al. 2008). These data therefore suggested that the anti-invasive and anti-metastatic activity of CBD was primarily due to down-regulation of Id1 gene expression. Indeed, ectopic expression of Id1 in breast cancer cells reversed the anti-invasive and anti-metastatic activity of CBD (McAllister et al. 2007; Murase et al. 2014). Overall, these data suggest that Id1 represents a potential biomarker for predicting whether CBD would be effective at inhibiting metastatic progression. Additional mechanisms in vivo that have been implicated in the anti-metastatic activities of CBD include the up-regulation of intercellular adhesion molecule-1 (ICAM-1) and tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) in lung cancer (Ramer et al. 2012). Besides CBD, other cannabinoids such as JWH-015, Win55,212-2, or a non-psychotropic CB_2 receptor-selective agonist, JWH-133, significantly inhibited breast and lung cancer cell progression and metastasis (Qamri et al. 2009; Caffarel et al. 2010; Nasser et al. 2011; Preet et al. 2011).

A recent study demonstrated that CBD inhibits breast cancer primary tumor growth and metastasis through direct inhibition of EGF/EGFR signaling and the tumor microenvironment (Elbaz et al. 2015). CBD inhibited the activation of NF-kB, EGFR, ERK, AKT as well as matrix metalloproteinase 2 and 9 in human breast cancer cells. Importantly, many of the major findings were confirmed in syngeneic and genetic mouse models of breast cancer. Additionally, CBD inhibited the recruitment of tumor-associated macrophages (TAM). In line with these findings, CBD inhibited the secretion of cytokines from the breast cancer cells that are known to attract TAM (Elbaz et al. 2015).

Suppression of Angiogenesis

Using human umbilical vein endothelial cells (HUVEC) in culture as a model, it was reported that CBD inhibited multiple processes involved in angiogenesis. Furthermore, this group significantly reduced angiogenesis in vivo in Matrigel sponges. Key down-stream targets inhibited by CBD in HUVEC cells included MMP-2 and -9, TIMP1, plasminogen

activator uPA, chemokines CXCL16 and IL-8, and growth factors enodothelin-1 and platelet derived growth factor-AA (Solinas et al. 2012). Moreover, CBD treatment led to a decrease in CD31 (vascularization marker) staining in tumor stroma in a mouse xenograft model where tumors were derived from subcutaneously implanted human lung cancer cells (Ramer et al. 2013).

Inhibition of Cancer Stem Cell Self-Renewal

Cancer stem cells are critical contributors to GBM therapeutic resistance and recurrence. It was demonstrated that CBD induced inhibition of patient-derived GSC self-renewal, and this effect was mediated by downregulation of expression levels of critical stem cell maintenance and growth regulators, such as Id1 and Sox2 (Soroceanu et al. 2013). More recently, it was shown that CBD inhibited self-renewal of GSCs in a ROS-dependent manner, by inhibiting phospho-STAT3 signaling as well as phopho-p38 MAP kinase pathway, both of which are key regulators of cancer stem cells (Singer et al 2015). Furthermore, the study demonstrated that GSCs treated with CBD underwent a proneural to mesenchymal transition exhibiting downregulation of proneural markers such as Olig2, Sox2 and upregulation of mesenchymal markers, such as CD44 (Singer et al 2015). A similar proneural to mesenchymal transition has been observed when GSCs were treated with radiation (Mao et al. 2013). In addition, the mesenchymal cells were shown to be markedly resistant to the effects of radiation. The CBD-dependent proneural to mesenchymal transition was reversed by anti-oxidant treatment, suggesting that ROS modulators may be used to prevent acquisition of a therapeutic resistant phenotype. Importantly, this study evaluated the efficacy of combining CBD with other small molecules that modulate system Xc and intracellular ROS, and showed a synergistic increase producing cell death when combining CBD and system Xc inhibitors (e.g., Erastin, Piperazine-erastin). System Xc has been shown to promote therapeutic resistance in several other cancers (Timmerman et al. 2013; Yoshikawa et al. 2013), therefore combining CBD with system Xc inhibitors may be efficacious in treating other malignancies in addition to glioblastoma.

Cannabinoids in Combination with First-Line Therapies

As reviewed above, treatment of cancer cells with cannabinoids leads to production of ROS, inhibition of Id1 gene expression and upregulation of autophagy-mediated cell death. Drugs that stimulate ROS have been shown to sensitize tumors to first-line agents (Wondrak 2009). Moreover, using genetic approaches to down-regulate Id1 expression has re-sensitized aggressive cancer cells to treatments with first-line therapies (Hu et al. 2009; Ponz-Sarvise et al. 2011). In breast cancer cells, where CBD is effective at downregulating Id1 expression, CBD enhanced the activity of the first-line agent, paclitaxel (Ward et al. 2014). Autophagy-mediated cell death mechanisms also contribute to efficacy of first-line therapies (Sui et al. 2013), and a primary mechanism for explaining the ability of a combination of THC and CBD to enhance the antitumor activity of temozolomide has indeed been upregulation of autophagy-mediated cell death (Torres et al. 2011). CBD and CBN have been shown to selectively decrease multidrug transporter expression leading to intracellular substrate accumulation and enhanced sensitivity of the cells to the cytotoxic actions of first-line therapies (Holland et al. 2006; Zhu et al. 2006; Holland et al. 2007). Two recent studies have investigated the ability of cannabinoids to enhance the effects of radiation in models of cancer. Emery et al. investigated the effects of THC and CBD to modulate the growth inhibitory effects of radiation in breast cancer cell lines in culture. Both cannabinoids did not alter the anti-proliferative effects of radiation (Emery et al. 2014). However, recent studies have evaluated the efficacy of CBD, THC or combinations thereof to enhance the anti-tumor effect of radiation using an immunocompetent orthotopic murine glioma model. Results showed that a combination of CBD and THC primed glioma cells to respond better to radiation suggesting a possible clinical benefit (Scott et al. 2014).

Another recent study investigated the ability of a variety of plant-derived cannabinoids to inhibit human prostate cancer (De Petrocellis et al. 2013). Of the cannabinoids tested, CBD was one of the most potent overall at inhibiting cell viability/proliferation. Xenograft tumors models utilizing human prostate cancer lines were then treated with a combination of CBD and the first-line agents taxotere and bicalutamide. A unique aspect of this study is that the mice were administered CBD orally, whereas a majority of cannabinoid antitumor investigations use systemic administration. As might be expected, there was heterogeneity in the response to treatment. CBD alone inhibited tumor progression in certain models but not in others, and enhanced the activity of first-line agents in two of the models but inhibited the efficacy of a first-line agent in one of the in vivo models.

Cannabinoids undergo significant first-pass metabolism after oral administration and this has been implicated in production of metabolites that reduce therapeutic activity (Perez-Reyes et al. 1973; Ohlsson et al. 1980). It would be of interest to determine whether the route of administration for delivery of cannabinoids is critical to antitumor activity and the enhancement of the effects of first-line therapies. There is no published research on the pharmacokinetics of cannabinoids

in relation to antitumor activity or sensitizing tumors to first-line agents. The antimetastatic activity of CBD during chronic systemic administration is observed in the range of 1–5 mg/kg (Ramer et al. 2010b; Murase et al. 2014). 15-25 mg/kg of CBD administered systemically and 10 mg/kg of CBD administered orally was needed to inhibit tumor progression in mouse xenograft models that more closely resemble primary tumor growth (Massi et al. 2004; Soroceanu et al. 2013; De Petrocellis et al. 2013). 3.7 mg/kg of CBD+3.7 mg/kg of THC delivered systemically and 100 mg/kg of CBD delivered orally were needed to sensitize tumors to first-line agents in mouse xenograft models that again more closely resemble primary tumor growth (Torres et al. 2011; De Petrocellis et al. 2013). Whether lower doses of CBD alone delivered orally would sensitize tumors to first-line agents has not been determined.

The significant psychotropic effects of THC would limit the use of high doses in humans. CBD however does not produce the psychotropic effects and has a low toxicity profile, therefore, using moderate to high doses of the drug is potentially feasible. The moderate effects of cannabinoids alone in mouse xenograft models that more closely resemble primary tumor growth suggest they may not be viable as a single drug therapy. This is not the case in models of metastasis, where the effects of cannabinoids appear to be more robust, particularly cannabinoid analogs (Murase et al. 2014). Cannabinoids however also sensitize, and in some cases resensitize, tumors to first-line agents (Torres et al. 2011). A cannabinoid drug treatment with a low toxicity profile that together produces direct antitumor activity and sensitizes tumors to existing first-line agents is an attractive therapeutic modality. This may explain why multiple groups are now in the process of initiating clinical trials to directly target aggressive cancer using cannabinoid-based therapeutics.

Since, the heterogeneous nature of tumors results in varying response to therapeutic treatments, a goal in treating cancer patients with cannabinoids would be to develop "signatures" that can predict which patient tumors will respond best to cannabinoids alone or in combination with first-line therapies. One of the first goals in this pursuit would be to determine the major pathways underlying treatment efficacy with non-psychoactive cannabinoids, in particular CBD.

Summary

Over the past decade researchers have refocused their efforts on the therapeutic potential of non-psychotropic cannabinoids in CS, in particular CBD. As presented in this review, the preclinical data strongly support the notion that non-psychoactive plant-derived CBs can act as direct inhibitors of tumor progression as well as enhance the activity of first-line therapies. While many anecdotal reports by cancer patients using various formulations of CS suggest significant efficacy, the lack of pure pharmacologically active compounds and legal restrictions surrounding schedule I drugs have delayed the clinical research that will ultimately determine whether cannabinoids are effective in the treatment of cancer beyond their proven palliative effects. It is promising to note that pharmaceutical companies have initiated clinical programs that include GMP-grade cannabinoids for targeting glioblastoma. Elucidation of the molecular pathways mediating non-psychoactive cannabinoid antitumor effects will be particularly important as the drugs move toward the clinic. A discussed earlier, certain tumors appear to be responsive to treatments while others are not. Currently, no markers have been discovered that can help oncologists identify which patients might benefit most from treatment with cannabinoids. Additional basic research will help lead to the development of cannabinoid-based therapies for the treatment of aggressive cancers and will also bring us closer to understanding the novel CB_1- and CB_2-independent component of the cannabinoid system that controls cancer progression.

Conflict of Interest The authors declare that they have no conflict of interest.

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