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Cannabinoids, Endocannabinoids and Cancer

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1. Introduction

1.1 Cannabinoid Function

Endocannabinoids are bioactive lipids that have a range of interesting activities mediated by two G-protein-coupled receptors (CB1 and CB2) and other putative targets [1-3]. The CB1 receptor is present in the central nervous system and mediates the psychotropic effects of exogenous cannabinoids such as Δ^9-tetrahydrocannabinol (THC), the active component of marijuana. In the brain, endocannabinoids and cannabinoids combine with CB1 cannabinoid receptors on axon terminals and regulate ion channel activity and neurotransmitter release [4]. Binding to the CB1 receptor is responsible for the analgesic activity of endocannabinoids as well as many other effects including locomotion and temperature control [5]. The CB2 receptor is present in inflammatory tissues and mediates the anti-inflammatory effects of endocannabinoids and plant-derived cannabinols [6]. Both the CB1 and CB2 receptors couple to Gi and reduce intracellular cAMP levels.

1.2 Biosynthesis and Degradation of Endocannabinoids

Endocannabinoids are synthesized "on demand" by post-synaptic cells and function as retrograde signaling molecules, diffusing back across the synapse to bind with pre-synaptic CB1 receptors, which reduces synaptic transmitter release [7]. The endocannabinoids are primarily produced biosynthetically from phospholipids [8]. The two primary endocannabinoids are anandamide (AEA) and 2-arachidonoylglycerol (2-AG). The most frequent biosynthetic route for AEA is through the transfer of arachidonic acid (AA) from the *sn*-1 position of phosphatidylcholine (PC) to the nitrogen atom of phosphatidylethanolamine (PE) by *N*-acyl transferase (NAT) to form N-arachidonoyl-phosphatidylethanolamine (NAPE) [8, 9]. NAPE is then converted into AEA in a one-step hydrolysis reaction catalyzed by the NAPE-specific phospholipase D (NAPE-PLD) (Figure 1) [10]. 2-AG is most frequently synthesized through the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) with AA on the *sn*-2 position to diacylglycerol (DAG) by phospholipase C-β (PLC-β). The DAG is then hydrolyzed to 2-AG by diacylglycerol lipase (DAGL) (Figure 2) [7, 11, 12].

The inactivation of endocannabinoids occurs by enzyme-catalyzed hydrolysis to AA; 2-AG is hydrolyzed by monoacylglycerol lipase (MAGL) and AEA is hydrolyzed by fatty acid amide hydrolase (FAAH) (Figure 3) [13, 14]. Endocannabinoids are also substrates for cycloxygenase-2 (COX-2), lipoxygenases (LOXs), and cytochromes P450 (CYP450s) and it is possible that these enzymes also play a role in controlling endocannabinoid levels by oxygenating 2-AG and AEA [15]. Induction of COX-2, LOXs, and CYP450s at sites of

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inflammation or in tumor cells could reduce the levels of naturally occurring anti-inflammatory and anti-proliferative lipid mediators. Furthermore, the products of endocannabinoid metabolism may exert effects that stimulate inflammation or tumor cell development by activating receptors that are distinct from classic receptors or by hydrolysis to products that are known to contribute to both inflammation and tumorigenesis [16]

Although the original focus of endocannabinoid biology was on neurological and psychiatric effects, these molecules are increasingly appreciated for their role in inflammation and cancer. The role of endocannabinoids in cancer has been implied by studies of the effects of exogenous cannabinoids, many derived from the plant *Cannabis sativa*, and synthetic compounds with activity at the CB1 and CB2 receptors (Figure 4). Endocannabinoids inhibit proliferation of cancer cells in culture and *in vivo* [17, 18]. In addition, endocannabinoids inhibit colonic inflammation, and deletion of CB receptors enhances colonic inflammation and cancer [19-21].

2. Cannabinoids and Cancer

2.1 Cannabinoid and Endocannabinoid Mediated Effects

Many laboratories have proposed that cannabinoids and endocannabinoids directly inhibit tumor growth *in vitro* and in animal tumor models through several different pathways. The inhibition of tumor growth and progression of several types of cancers including glioma, glioblastoma, breast cancer, prostate cancer, thyroid cancer, colon carcinoma, leukemia, and lymphoid tumors have been demonstrated by natural and synthetic cannabinoids, endocannabinoids, endocannabinoid analogs, endocannabinoid transport inhibitors, and endocannabinoid degradation inhibitors. Several different mechanisms have been implicated in the anti-tumorigenic actions of endocannabinoids and include cytotoxic or cytostatic effects, apoptosis induction, and anti-metastatic effects such as inhibition of neo-angiogenesis and tumor cell migration [22]. These effects are dependent on CB1, CB2, transient receptor potential vanilloid type 1 (TRPV1), or are receptor-independent based on the cannabinoid or endocannabinoid and the tissue or tumor cell.

Endocannabinoid levels are finely modulated under physiological and pathological conditions. A transient increment appears to be an adaptive reaction to restore homeostasis when this is acutely and pathologically perturbed. However, in some chronic conditions, the alteration of the endocannabinoid system seems to contribute to the progress and symptoms of the disease. In particular, several different types of cancer have abnormally regulated endocannabinoid systems.

2.2 Changes in Endocannabinoid Tone and Signaling in Tumors

Elevated levels of AEA and 2-AG have been reported in several types of tumors when compared with their normal counterparts, specifically in glioblastoma, meningioma, pituitary adenoma, prostate and colon carcinoma, endometrial sarcoma, and in highly invasive human tumor cells [22-27]. The enzymes that synthesize and metabolize the endocannabinoids control their effects by modulating the localized concentrations. A correlation between endocannabinoid metabolizing enzymes, FAAH (for AEA) and MAGL (for 2-AG), and cancer has been investigated in prostate adenocarcinomas. MAGL is elevated in androgen-independent versus androgen-dependent human prostate cancer cell lines, and pharmacological or RNA-interference disruption of MAGL impairs prostate cancer aggressiveness [138]. An increase of FAAH expression in prostate cancer compared to normal prostate tissue samples has been reported [29]. In contrast, in human patients with pancreatic ductal adenocarcinomas a correlation between high FAAH and MAGL levels and survival has been observed [30].

Cannabinoid receptor levels are a major determinant of the effects of endocannabinoids. CB1 receptors show an increase in expression when treated with agonists in several cancer cell lines; however, in normal tissue these agonists decrease CB1 receptor expression [31]. This difference in expression may be a mechanism by which normal cells are protected from the pro-apoptotic and anti-proliferative effects of cannabinoid agonists [22]. It has been shown that THC induces apoptosis in several human cancer cell lines while sparing non-transformed cell lines [32-35].

Cannabinoid receptor expression in tumor cells versus normal cells is an important consideration. Although the mechanisms by which cannabinoid receptor expression is modulated have not been fully investigated, several important studies have revealed critical interactions between cannabinoid receptor expression and cancer. For example, it has been shown that THC induces a CB2 receptor-dependent transcription of the CB1 gene in T cells and T cell lymphoma lines [36]. Up-regulation of the CB1 gene is mediated by IL-4 release and activation of the transcription factor STAT6 [36]. It has been reported that oral administration of specific *Lactobacillus* strains induce CB2 receptor expression in colonic epithelial cells through the NF-κB pathway [37]. In addition, CB1 receptor expression is induced by 17-β-estradiol in human colon cancer cells through an estrogen-receptor dependent mechanism [38]. Chromatin immunoprecipitation studies have demonstrated that the CB1 gene is a transcriptional target of PAX3/FKHR, a chimeric transcription factor found in alveolar rhabdomyosarcoma, where the CB1 receptor is highly overexpressed [39]. Another theory has been presented that alternative spliced isoforms of CB1 (CB1a and CB1b) could reflect differences in its functionality in normal and malignant tissues [40].

The association of CB receptor expression with tumor malignancy and disease outcome in cancer has been studied in several settings. These studies suggest that the role of CB1 and CB2 receptor expression in relation to disease prognosis and outcome is dependent on the specific cancer type. Analyses of astrocytomas demonstrate that 70% of the tumors express CB1 and/or CB2 and the extent of CB2 expression correlates with tumor malignancy [41]. In gliomas, a higher expression of CB2 compared to CB1 has been reported and is related to tumor grade [41]. In addition to tumors, tumor-associated endothelial cells exhibit immunoreactivity for CB receptors similar to that observed in tumor cells [42]. Increased expression of CB1 has been reported in mantle cell lymphoma and of both CB1 and CB2 in non-Hodgkin lymphoma as compared to reactive lymph nodes [43, 44]. In contrast, a greatly reduced expression of CB1, but not CB2, was found in colon carcinoma compared with adjacent normal mucosa [19].

In breast cancer, a correlation between CB2 expression and the histological grade of the tumors as well as other markers, such as estrogen and progesterone receptor levels and the presence of the ERBB2/HER-2 oncogene, has been observed [45]. In prostate cancer, CB1 receptor expression by the human prostate cancer cell lines LNCaP (androgen-sensitive), DU145 and PC3 (androgen-independent) are higher than that seen in normal human prostate epithelial cells [46]. This was confirmed in prostate carcinoma specimens where expression of the CB1 and TRPV1 receptors are up-regulated and correlate with increasing tumor grades [47]. It has also been shown that the level of CB1 in tumor tissue is associated with disease severity at diagnosis and outcome [48]. In pancreatic tumors high CB1 receptor expression is associated with a shorter survival time (median 6 months) than low CB1 expression (median 16 months) in humans [30]. In contrast, in hepatocellular carcinoma, over-expression of CB1 and CB2 receptors are correlated with improved prognosis in humans [49].

2.3 Cannabinoid Receptor-Independent Effects

In addition to signaling through cannabinoid receptors, cannabinoids, in particular anandamide and cannabidiol, have CB receptor-independent effects. AEA and other lipids have been shown to activate TRPV1 [50]. AEA has been shown to induce neuroblastoma, lymphoma, and uterine cervix carcinoma cell death through vanilloid receptors [51, 52]. In addition, inhibition of cancer cell invasion through TIMP1, an inhibitor of metalloproteinases, by methanandamide (AM-356), a hydrolysis resistant AEA analog, is mediated by TRPV1 [45]. It has also been proposed that lipid rafts, membrane domains rich in sphingolipids and cholesterol, mediate AEA effects through CB1 signaling [53, 54]. In cholangiocarcinoma, the anti-proliferative and pro-apoptotic action of AEA is facilitated by lipid raft stabilization, ceramide accumulation, and recruitment of FAS and FAS ligand into lipid rafts [55].

Another cellular protein that may be important in CB receptor-independent cell death induced by endocannabinoids is COX-2. COX-2 metabolizes AA to prostaglandins (PGs) and elevated levels of both COX-2 and PGs have been measured in neoplastic tissues. COX-2 is also capable of metabolizing AEA to prostaglandin ethanolamides (PG-EAs) and 2-AG to glycerol prostaglandins (PG-Gs) [56, 57]. AEA inhibits growth and induces apoptosis in the colon carcinoma cell lines HT29, a moderate COX-2 expressor, and HCA7/C29, a high COX-2 expressor [57]. AEA also inhibits growth and induces apoptosis in COX-2 transfected tumorigenic keratinocytes, but has little effect on the very low COX-2 expressing colon carcinoma cells SW480 and HaCaT keratinocytes [58]. Apoptosis induced by AEA in human neuroglioma cells is COX-2 mediated and not affected by antagonists of the cannabinoid receptors or TRPV1 [59]. In human neuroblastoma and C6 glioma cells AEA induces apoptosis through a vanilloid receptor mediated increase in intracellular calcium concentration, which activates COX-2, releases cytochrome *c* and activates caspase 3 [52].

An important molecule for studying cannabinoid receptor-independent effects is cannabidiol. Cannabidiol is a cannabinoid analog that has no activity at CB1 or CB2 receptors and lacks psychotropic effects. Cannabidiol has been shown to inhibit glioma and breast tumor growth *in vitro* and *in vivo* through induction of apoptosis and inhibition of cell migration and angiogenesis, with these effects being independent of CB and TRPV1 receptor activity [60-62]. Cannabidiol reduces the invasiveness of breast cancer cells by inhibiting Id-1, an inhibitor of basic helix-loop-helix transcription factors involved in tumor progression, at the promoter level [63]. A quinone analog of cannabidiol, HU-331, a highly specific inhibitor of topoisomerase II, has been reported to have high efficacy against human cancer cell lines *in vitro* and against tumor grafts in nude mice [64]. HU-331 also inhibits angiogenesis by directly inducing apoptosis of vascular endothelial cells without changing the expression of pro- and anti-angiogenic cytokines and their receptors [65].

Cannabinoids may also interfere with the ability of lysophosphatidylinositol (LPI) to bind to GPR55. LPI induces cancer cell proliferation through GPR55 activation by triggering the initiation of ERK, AKT, and calcium mobilization cascades [66]. The activation of these cell proliferation cascades by GPR55 has been verified using siRNA to block LPI signaling through GPR55 [66]. In addition, pretreatment of breast and prostate cancer cells with cannabidiol or Rimonabant (SR141716A), a CB1 antagonist that also binds to GPR55, blocks the ability of LPI to induce cell proliferation through GPR55 [66].

3. CB1 and CB2 Mediated Anti-proliferative and Apoptotic Effects of Cannabinoids

3.1 Cannabinoid Modulation of Cell Cycle Regulation

Cannabinoids have been shown to cause cell cycle arrest in various cancer cell lines. AEA arrests the proliferation of MDA-MB-231 human breast cancer cells in the S phase of the cell cycle through a loss in Cdk2 activity, up-regulation of p21waf, and a reduced formation of the active complex cyclin E/Cdk2 [67]. AEA arrests cells in S phase through activation of Chk1 and Cdc25A proteolysis, which prevents activation of Cdk2 through dephosphorylation of Thr14/Tyr15, critical inhibitory residues on Cdk2 [67]. THC inhibits breast cancer cell proliferation by blocking the progression of the cell cycle in the G2/M phase through the down-regulation of Cdc2 in a CB2 receptor-dependent manner [68]. However, CB2-selective antagonists significantly, but not totally, prevent these effects, suggesting a contribution of a CB2 receptor-independent mechanism [68]. The CB1 and CB2 agonist WIN-55,212-2 causes LNCaP human prostate cancer cell arrest in the G0/G1 phase of the cell cycle [69]. Activation of ERK1/2, induction of p27/KIP1, and inhibition of cyclin D sustain the arrest [69].

Importantly, G0/G1 arrest enhances the Bax/Bcl-2 ratio and activates caspases, resulting in an induction of apoptosis. WIN-55,212-2 treatment of LNCaP cells also causes a dose-dependent decrease in the expression of cyclin D1, cyclin D2 and cyclin E, as well as cdk2, cdk4 and cdk6, pRb and its molecular partner, the transcription factor E2F [69]. WIN-55,212-2 causes a dose-dependent decrease in the protein expression of DP-1 and DP-2, which form heterodimeric complexes with E2F essential for activity [69]. THC administration also elicits G0/G1 cell cycle blockade in glioblastoma cells through the suppression of E2F1 and Cyclin A and the up-regulation of the cell cycle inhibitor p16(INK4A) [70].

3.2 Induction of Apoptosis by Cannabinoids

THC has been shown to induce apoptosis via CB1 inhibition of RAS-MAPK and PI3K-AKT survival signaling and induction of BAD-mediated apoptosis in colorectal cancer cells [71]. CB1 also reduces cyclic AMP-dependent protein kinase A signaling leading to down-regulation of the anti-apoptotic factor survivin [45]. Survivin over-expression is associated with poor clinical outcomes and reduced tumor apoptosis in patients with colorectal cancer [73, 74]. Survivin is an attractive target for pharmacological modulation because it is over-expressed in most human tumors but is present in very small amounts in normal adult tissues [74]. A direct link between CB1 activation and decreased survivin expression has been established through treatment of SW-480 cells with AM-356, a CB1 receptor agonist [19].

Activation of CB1 or CB2 receptors has been shown to stimulate *de novo* synthesis of ceramide in human tumors including glioma, leukemia, and pancreatic, and DLD-1 and HT29 colorectal cancer cells [75-77]. Ceramide is a pro-apoptotic lipid that causes up-regulation of the stress protein p8 and several downstream stress-related genes expressed in the endoplasmic reticulum including ATF-4, CHOP, and TRB3 [78]. Ceramide also causes prolonged activation of the Raf1/extracellular signal-regulated kinase cascade, inhibition of Akt, c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinase [33,59,79-83]. In DLD-1 and HT29 colorectal cancer cells, CB1 and CB2 receptor activation leads to increased ceramide levels, whereas CB1 and CB2 receptor-induced apoptosis is prevented by the pharmacologic inhibition of *de novo* ceramide synthesis [77]. The synthesis of ceramide appears to be mediated in part by TNFα; knockdown of TNFα abrogates the ceramide increase and prevents the apoptotic effect induced by cannabinoid receptor activation [77].

A role for Bcl-2 family members, such as Bad, has also been hypothesized in cannabinoid-dependent apoptosis [81]. Pro-apoptotic effects may rely also on a CB1 receptor-independent stimulation of sphingomyelin breakdown [84]. In lymphoma and leukemia cell lines, CB agonists such as THC and WIN-55,212-2 induce CB-dependent apoptosis through ceramide accumulation and caspase activation via the p38MAPK signaling pathway, down-regulation of the RAF1/MAPK pathway, and translocation of BAD to mitochondria [85, 86]. A common event in cannabinoid-induced apoptosis is the depolarization of mitochondria via cytochrome c release [85-87]. CB agonists have been reported to be mitochondrial inhibitors, since they decrease oxygen consumption and mitochondrial membrane potential while increasing mitochondrial hydrogen peroxide production, thus inducing apoptosis [88].

3.3 Inhibition of Tumor Proliferation by Cannabinoids

Cannabinoids inhibit the proliferation of various tumor cells through the inhibition of proliferative and oncogenic pathways such as adenylyl cyclase and cAMP/protein kinase A pathway, cell cycle blockade with induction of the cyclin-dependent kinase inhibitor $p27^{kip1}$, decrease in epidermal growth factor receptor (EGF-R) expression and/or attenuation of EGF-R tyrosine kinase activity, decrease in the activity and/or expression of nerve growth factor, prolactin or vascular endothelial growth factor tyrosine kinase receptors [32, 89-93].

AEA inhibits breast cancer cell proliferation through down-regulation of the prolactin receptor, *brca1* gene product, and the high affinity neurotrophins receptor *trk* [89, 93]. The anti-proliferative effect of AEA is proportional to the degree of hormone dependency of the cell lines and the mechanism relies on the inhibition of the cAMP-dependent PKA pathway [93]. Several intraepithelial or invasive prostatic cancers show increased expression of EGF-R, EGF and transforming growth factor α (TGFα) [91]. AEA inhibits the EGF-induced proliferation of DU145 and PC3 prostate cancer cells, as well as of androgen-stimulated LNCaP cells, via G1 arrest and down-regulation of EGF-R [91]. These effects are CB1-mediated [91]. Similar growth arrest and receptor modulation by AEA are observed in prolactin and nerve growth factor-stimulated DU145 cells [92-94]. Treatment of LNCaP cells with WIN-55,212-2 results in decreased proliferation, androgen receptor expression, VEGF protein expression, and secreted levels of PSA, a glycoprotein androgen receptor-regulated protein that is a marker of prostate cancer progression [46]. The antagonistic effect of endocannabinoids on growth factor-induced proliferation has also been reported in glioma [95].

3.4 Inhibition of Tumor Neovascularization by Cannabinoids

Cannabinoids have been shown to inhibit tumor growth by lowering vascular density in tumors. Several cannabinoids that bind to CB1 and/or CB2 receptors, including WIN-55,212-2, HU-210, JWH-133, and THC, have been shown to inhibit vascular endothelial cell survival and migration as part of their antiangiogenic action [96]. Cannabinoids cause a lower distribution of CD31-positive cells, a common angiogenesis marker, in experimental tumor xenografts from glioma, melanoma and nonmelanoma skin cancer, and lung tumor cells [32, 96-98]. Met-fluoro-anandamide (Met-F-AEA), a metabolically stable analog of AEA, has been demonstrated to reduce the sprout number and length of endothelial cell spheroids, inhibit capillary-like tube formation *in vitro*, and suppress angiogenesis in an *in vivo* chick chorioallantoic membrane assay [99]. Furthermore, experimental tumors from animals treated with cannabinoids have been shown to exert a vascular network that is small, undifferentiated, and impermeable giving tumors a paler appearance when compared to controls [90, 96].

In addition to the direct inhibition of vascular endothelial cell migration and survival, cannabinoids decrease the expression of proangiogenic factors in tumors. Several studies

have revealed that cannabinoids have an effect on the expression of VEGF, which is one of the major cancer cell-released chemoattractants in tumor neovascularization [100]. Met-F-AEA has been shown to decrease levels of VEGF and VEGFR-1 in K-ras-transformed thyroid cells and in experimental tumors of xenografted nude mice [90]. In skin carcinoma mouse models, JWH-133 and WIN-55,212-2 inhibit vascular hyperplasia, which is associated with a reduced mRNA expression of VEGF [32]. THC suppresses the release of VEGF in non–small cell lung cancer (NSCLC) cells [98]. JWH-133 mediates decreased expression of proangiogenic factors related to VEGF signaling in mouse gliomas including VEGF-A, VEGF-B, and hypoxia-inducible factor 1α (HIF-1α), which is the main transcription factor responsible for VEGF expression [101]. JWH-133 down-regulates connective tissue growth factor and heme oxygenase-1, genes known to be regulated by VEGF, as well as the VEGF-related factors, inhibitor of differentiation-3 (Id-3), midkine, and the angiopoietin receptor tyrosine kinase with immunoglobulin-like and epidermal growth factor (EGF)-like domains 1 (Tie-1) [101, 102]. In contrast, JWH-133 induces the expression of type I procollagen 1α chain, a matrix metalloproteinase (MMP) substrate related to matrix remodeling during angiogenesis [101, 103].

In vivo experiments have also demonstrated that JWH-133 and WIN-55,212-2 decrease the mRNA levels and autophosphorylation activity of EGFR in skin tumors [32]. Cannabinoids diminish the expression of angiopoietin-2 (Ang-2) and placental growth factor (PlGF) along with the appearance of narrow capillaries and a decrease of blood vessel size [32]. JWH-133 down-regulates Ang-2, which supports the formation of mature blood vessels, in gliomas and astrocytomas [96, 101].

Angiogenesis involves several proteolytic enzymes. THC down-regulates the proangiogenic factor MMP-2 in human tumor samples from recurrent glioblastoma multiforme and in nude mice xenografted with the C6.9 subclone from rat glioma C6 cells [104]. Importantly, THC does not alter the expression of MMP-2 in the C6.4 subclone, a nonresponder, from rat glioma C6 cells [104]. THC and methanandamide decrease MMP-2 expression *in vitro* in cervical cancer cells accompanied by a reduced invasiveness of the cancer cells [45]. JWH-133 also decreases MMP-2 expression *in vivo* in glioma xenografts and impairs tumor vasculature [96]. Met-F-AEA also inhibits MMP-2 activity in endothelial cells [99].

The effects of cannabinoids on several antiangiogenic factors have also been studied. WIN-55,212-2 and JWH-133 do not have an effect on the expression of thrombospondin-1 and -2, multidomain matrix glycoproteins that inhibit neovascularization, in nude mice xenografted with melanoma carcinoma cells [32]. The effects of cannabinoids on the expression of TIMP-1, an inhibitor of angiogenesis, are dependent on the specific cancer cell line used [103]. In human cervical and lung cancer cells, cannabinoids up-regulate TIMP-1 expression and are anti-invasive [45]. In contrast, THC down-regulates TIMP-1 in glioma cell lines and in human tumor samples from recurrent glioblastoma multiforme patients [105]. JWH-133 also down-regulates TIMP-1 in nude mice xenografted with C6.9 glioma cells [105]. The cannabinoid derivative HU-331 is antiangiogenic through a different mechanism. HU-331 inhibits angiogenesis by directly inducing apoptosis of vascular endothelial cells without modulating the expression of pro- and antiangiogenic factors and their receptors [65].

3.5 Effects of Cannabinoids on Tumor Cell Migration

Tumor cell migration is an important step for the spread of cancer [106]. As an initial step, the primary tumor has to enter lymphatic or blood vessels. Migration of cancer cells is initiated by paracrine or endocrine chemoattractants but is also affected by neurotransmitters and other factors.

Among the chemoattractants that trigger migration, cell growth, proliferation, and differentiation, EGF and its receptor, EGFR, play a pivotal role. THC elicits a decrease of EGF-induced migration of NSCLC cells in scratch wound and Transwell migration experiments but has no effect on basal migration [98]. As described earlier, THC action modulates intracellular signaling events downstream of EGFR, such as inhibition of mitogen-activated protein kinases and protein kinase B (Akt) activity [98]. The impact of cannabinoids on EGFR activation appears to be cell type specific. In glioma and lung carcinoma, cannabinoid receptor agonists induce cell proliferation through cannabinoid-induced EGFR signal transactivation [107]. In skin tumors *in vivo*, WIN-55,212-2 and JWH-133 inhibit EGFR activation [32]. AEA inhibits EGFR expression and inhibits EGFR-induced proliferation, through CB1 signaling, in prostate cancer cells [91]. Human astrocytoma cells have no change in EGFR tyrosine phosphorylation when treated with cannabinoids [108].

Neurotransmitters also play a role in regulating cell migration [109]. Cannabinoids have an inhibitory action on norepinephrine-induced cancer cell migration [110]. AEA, HU-210, and the AEA analog docosatetraenylethanolamide (DEA) block the migration of colon carcinoma cells with low CB2 receptor expression while JWH-133 has no effect [110]. AEA and HU-210 activate both cannabinoid receptors, DEA acts as a CB1 receptor agonist, and JWH-133 acts as a CB2 receptor agonist; thus, in colon carcinoma cells, CB1 likely mediates the antimigratory actions of cannabinoids [110].

The pathways involved in CB1-receptor dependent antimigratory effects have been explored in some depth. In breast cancer cells, Met-F-AEA causes a CB1 receptor-dependent antimigratory effect involving the RhoA/Rho-associated coiled coil-containing kinase (RhoA-Rock) system [111, 112]. Met-F-AEA inhibits the activity of the GTPase, RhoA, and causes RhoA to translocate from the cell membrane to the cytosol, which causes alterations in the actin cytoskeleton [112].

Mast cells are a source of chemoattractants and are possible targets of cannabinoids [113]. Cancer cell migration initiated by mast cells is down-regulated by 2-AG and WIN-55,212-2 in the scratch wound healing assay in a CB1-receptor dependent manner [113]. Human glioma cell migration is inhibited by cannabidiol in a receptor-independent manner, as evidenced by the failure of cannabinoid receptor antagonists and pertussis toxin to reverse the antimigratory action of cannabidiol [61]. AM-356 and THC do not affect the basal migration of human cervical and lung cancer cells, implicating a cell type-specific or chemoattractant-dependent regulation of migration by cannabinoids [45]. Thus, cannabinoids are antimigratory in some cancer cell lines but the underlying signaling pathways are not fully elucidated.

3.6 Influence of Cannabinoids on Cancer Cell Adhesion

The adhesive interaction of tumor cells with the surrounding microenvironment is a critical factor in their growth, migration and metastasis. Matrix proteins such as integrins, cadherins, selectins, and cell adhesion molecules of the immunoglobulin superfamily (IgSF CAMs) are integral to the adhesion of tumor cells to the extracellular matrix (ECM). Cannabinoids have been shown to have various effects on the adhesion of tumors cells to the ECM. Met-F-AEA selectively reduces the adhesion of human breast cancer cells to the ECM component collagen type IV in a CB1 receptor-dependent manner *in vitro*, but has no effect on adhesion to fibronectin and laminin [111]. Met-F-AEA does not affect the expression of integrins but it does decrease their affinity for collagen through suppression of phosphorylation of the focal adhesion kinase (FAK) and the pro-oncogenic tyrosine kinase Src [111]. HU-210 does not have a direct effect on FAK phosphorylation in murine neuroblastoma cells [114].

HU-210 instead phosphorylates FAK-related nonkinase (FRNK), which inhibits FAK activity, in a CB1 receptor-dependent manner [114-117].

Cannabinoids influence IgSF CAMs. WIN-55,212-2 blocks the interleukin 1 (IL-1)-induced up-regulation of intercellular cell adhesion molecule 1 and vascular cell adhesion molecule 1- two IgSF CAMs- in human glioblastoma and lymphoma cells in a cannabinoid receptor-independent manner [118]. WIN-55,212-2 produces this effect by inhibiting IL-1-induced activation of the transcription factor NFκB, a key regulator in the expression of cell adhesion molecules [118].

3.7 Effects of Cannabinoids on Tumor Cell Invasion

Cancer cell invasion is one of the crucial events in local spreading, growth, and metastasis of tumors. 2-AG inhibits the invasion of androgen-independent prostate cancer cells through CB1 receptor activation [26]. However, the precise mechanism leading to decreased invasiveness by cannabinoids has not been fully elucidated. Several investigations have provided insight into how cannabinoids may achieve their anti-invasive action.

Cannabinoids have been shown to modulate the MMP system, which, in part, leads to their anti-invasive action. MMPs degrade ECM components, an important function in tumor invasion, metastasis, and angiogenesis [119, 120]. MMPs, specifically MMP2- and MMP-9, facilitate tumor invasion by proteolytic degradation of major basement membrane components, such as type IV collagen, laminin, and nidogen [119]. MMP proteolytic activity is inhibited by TIMPs that bind noncovalently in a 1:1 stoichiometric ratio to active MMPs. Higher ECM degradation in invasive and metastatic tumor cells can result from an increased level of MMPs and a decreased level of TIMPs, which causes increased proteolytic activity.

Cannabinoids have been shown to have a direct effect on the MMP system. JWH-133 decreases the expression and activity of MMP-2 in mice xenografted with a rat glioma cell line and human grade IV astrocytoma cells [96]. Met-F-AEA also inhibits MMP-2 activity, leading to an antiangiogenic effect [99]. In addition, THC inhibits MMP-2 expression and cell invasion in glioma cells [104]. Modulation of MMP-2 expression by RNA interference and cDNA overexpression reveals that down-regulation of MMP-2 plays a critical role in THC-mediated inhibition of cell invasion [104]. Cannabinoid-induced inhibition of MMP-2 expression and cell invasion is prevented by blocking ceramide biosynthesis and by knocking down the expression of the stress protein p8 [104]. A concentration-dependent inhibition of MMP-2 by AM-356 and THC in cervical carcinoma cells also occurs, however, it is independent of CB receptor and TRPV1 activation [45].

There is a correlation between high cancer invasiveness and decreased TIMP-1 expression; in addition, the anti-invasive action of several drugs has been associated with elevated TIMP-1 levels [121-127]. In contrast, TIMP-1 up-regulation is associated with poor patient prognosis because TIMP-1 has MMP-independent antiapoptotic properties [128]. The anti-invasive action of AM-356 and THC depends on the induction of TIMP-1 expression in cervical carcinoma and lung cancer cells [45]. The expression of TIMP-1 is stimulated by CB1 and CB2 receptor activation and, in the case of AM-356, by activation of TRPV1 [45]. TIMP-1 expression has also been shown to be modulated by the p38 mitogen-activated protein kinase and the extracellular regulating kinases 1 and 2 (ERK1/2) [45]. In glioma cell lines and primary tumor cells from glioblastoma multiforme tissues, TIMP-1 expression is inhibited by cannabinoids [105]. Instead, the cannabinoid-induced apoptosis is dependent on *de novo* synthesis of ceramide [105]. Thus, cannabinoid action on TIMP-1 expression and the subsequent impact on tumorigenesis depends on tumor type.

3.8 *In Vivo* Effects of Cannabinoids

In vivo studies demonstrate that cannabinoids reduce tumor growth and metastasis as well as cell proliferation and angiogenesis in mice. THC decreases tumor size, number of tumor and lung metastases, and inhibits both cell proliferation and angiogenesis in an animal model of metastatic breast cancer [129]. This inhibition of cell proliferation involves CB2 but not CB1 receptors [129]. The CB2 agonist JWH-133 reduces the size and number of tumors, number and size of lung metastases, inhibits cell proliferation, and decrease angiogenesis in mice injected with different breast cancer cell lines [129, 130]. In CB-17 immunodeficient mice injected with MDA-MB-231 cells, the mixed CB1/CB2 agonist WIN55,212-2 reduces tumor size, decreases the number and size of lung metastases, inhibits proliferation and reduces angiogenesis through activity at both the CB1 and CB2 receptors [130]. Cannabidiol reduces tumor growth and size and decreases the number of lung metastases in mice injected with MDA-MB-231 or 4T1 breast cancer cell lines [62, 131]. AM-356 reduces the number and size of lung tumor nodules in mice injected with TSA-1 mammary carcinoma cell line through CB1 activity [111]. In contrast, the CB1 antagonist Rimonabant decreases tumor size in mice injected with MDA-MB-231 cancer cells [132]. Direct injection of the preferential CB2 agonist JWH-015 reduces tumor growth in athymic nude male mice injected with PC-3 prostate carcinoma cells and this reduction of growth is inhibited by the CB2 receptor antagonist SR144528 [133].

Although many studies have found beneficial effects of cannabinoids in the treatment of cancer, there are several conflicting reports. Systemic administration of THC increases the local tumor size and the number and size of metastasis in mice injected with 4T1 tumor cells into the rear footpads [134]. This effect may be due to the fact that THC suppresses the anti-tumor immune response, which is mediated by CB2 [134]. SCID-NOD mice, which are devoid of anti-tumor immune responses, do not exhibit increases in tumor size or metastasis following THC administration [134].

4. Conclusions

Cannabinoids exert a number of interesting effects that are dependent on the cell line or tumor type. Synthetic cannabinoids and the endocannabinoid system are implicated in inhibiting cancer cell proliferation and angiogenesis, reducing tumor growth and metastases, and inducing apoptosis. Some studies suggest that abnormal regulation of the endocannabinoid system may promote cancer by fostering physiological conditions that allow cancer cells to proliferate and migrate. For this reason, the endocannabinoid is an attractive target for pharmacological intervention in the treatment of cancer. Modulation of the endocannabinoid system to treat cancer may provide a targeted treatment of cancer, which has been shown in several studies that demonstrated selective action of cannabinoids on tumor cells while not having effects on normal cells.

The endocannabinoid system is involved in a complex set of signaling pathways including activity at the CB1, CB2, TRPV1, and GPR55 receptors, and through receptor-independent actions. The complexity of the signaling pathways involved in endocannabinoid action both in normal and malignant tissues offer a significant research obstacle, however, several important pathways have been elucidated. These include modulation of pathways critical to cell proliferation, cell cycle, and apoptosis. The diversity of receptors and signaling pathways that the endocannabinoid system modulates offers an interesting opportunity for the development of specific molecules to perturb the system selectively, as has already been achieved in the development of agonist and antagonists of the CB1, CB2, TRPV1, and GPR55 receptors. In addition, recent work has revealed that COX-2, which is involved in the progression of several types of cancer, modulates endocannabinoid tone at sites of

inflammation [135]. The oxygenation of endocannabinoids by COX-2 or other enzymes may also play a critical role in the influence of endocannabinoids on cancer.

Although there is a strong set of data *in vitro*, in cellular model systems, and in mouse model systems, there is a dearth of clinical data on the effects of cannabinoids in the treatment of cancer in humans. This fact is quite surprising considering the large library of compounds that have been developed and used to study the effects of cannabinoids on cancer in model systems. Despite the lack of preclinical and clinical data, there is a strong agreement that pharmacological targeting of the endocannabinoid system is emerging as one of the most promising new methods for reducing the progression of cancer. In particular, combination therapy utilizing both traditional chemotherapeutics and molecules targeting the endocannabinoid system may be an excellent next generation treatment for cancer.

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Abbreviations

THC	Δ^9-tetrahydrocannabinol
AEA	anandamide
2-AG	2-arachidonoylglycerol
AA	arachidonic acid
PC	phosphatidylcholine
PE	phosphatidylethanolamine
NAT	by *N*-acyl transferase
NAPE	N-arachidonoyl-phosphatidylethanolamine
NAPE-PLD	NAPE-specific phospholipase D
PIP_2	phosphatidylinositol-4,5-bisphosphate
DAG	diacylglycerol
PLC-β	phospholipase C-β
DAGL	diacylglycerol lipase
MAGL	monoacylglycerol lipase
FAAH	fatty acid amide hydrolase
COX-2	cycloxygenase-2
LOXs	lipoxygenases
CYP450s	cytochromes P450
TRPV1	transient receptor potential vanilloid type 1
AM-356	methanandamide
PGs	prostaglandins
PG-EAs	prostaglandin ethanolamides
PG-Gs	glycerol prostaglandins

LPI	lysophosphatidylinositol
EGF-R	epidermal growth factor receptor
TGFα	transforming growth factor α
Met-F-AEA	Met-fluoro-anandamide
NSCLC	non–small cell lung cancer
HIF-1α	hypoxia-inducible factor 1α
Id-3	inhibitor of differentiation-3
Tie-1	angiopoietin receptor tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domains 1
MMP	matrix metalloproteinase
Ang-2	angiopoietin-2
PlGF	placental growth factor
Akt	protein kinase B
DEA	docosatetraenylethanolamide
RhoA-Rock	RhoA/Rho-associated coiled coil-containing kinase
IgSF CAMs	cell adhesion molecules of the immunoglobulin superfamily
ECM	extracellular matrix
FAK	focal adhesion kinase
FRNK	FAK-related nonkinase
IL-1	interleukin 1
ERK1/2	extracellular regulating kinases 1 and 2

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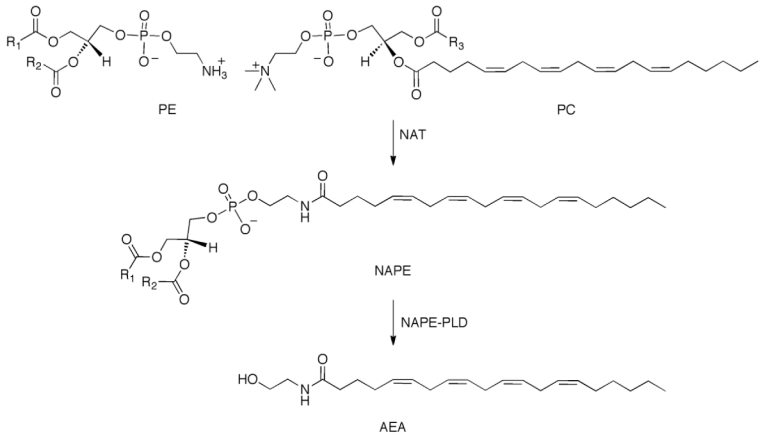

Figure 1. Biosynthesis of AEA

NAT catalyzes the transfer of AA from PC to the ethanolamine of PE to from NAPE. NAPE is then hydrolyzed by NAPE-PLD to form AEA.

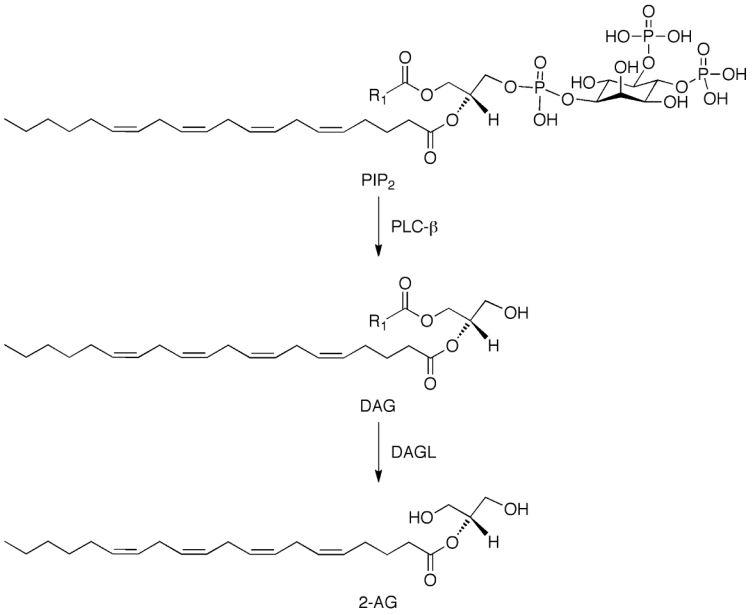

Figure 2. Biosynthesis of 2-AG

PIP_2 is hydrolyzed by PLC-β to form DAG. DAGL then hydrolyzes DAG to form 2-AG.

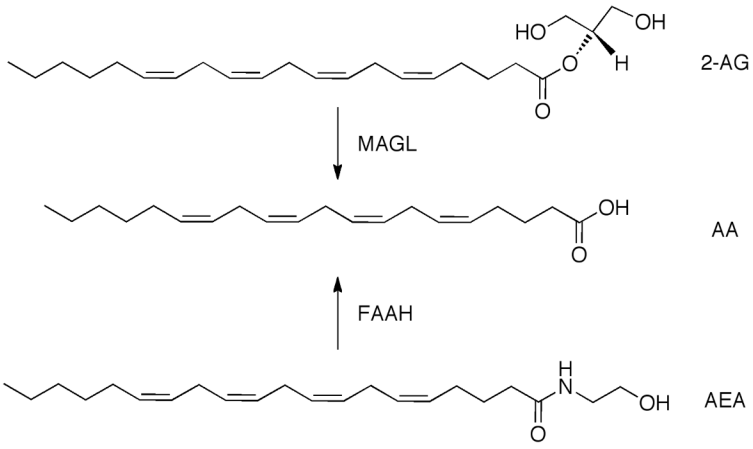

Figure 3. Metabolism of 2-AG and AEA

The endocannabinoids are primarily metabolized by hydrolysis to AA. 2-AG is hydrolyzed by MAGL and AEA is hydrolyzed by FAAH.

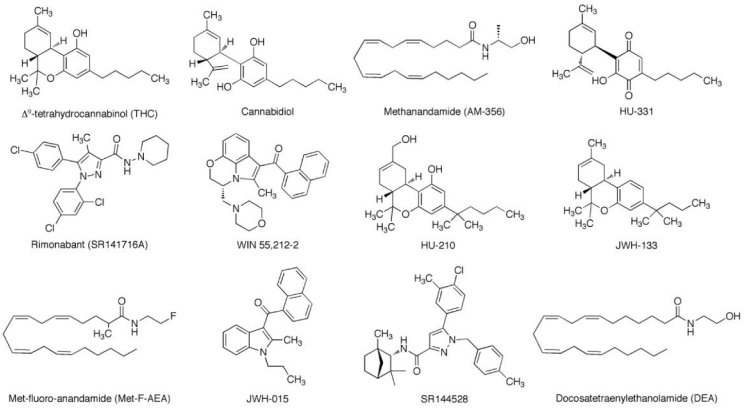

Figure 4.
Structures of compounds used to study the endocannabinoid system.