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Review

# The endocannabinoid system in cancer—Potential therapeutic target?

Jenny Flygare, Birgitta Sander *

*Department of Laboratory Medicine, Divison of Pathology, F46, Karolinska Institutet and Karolinska University Hospital Huddinge, SE 141 86 Stockholm, Sweden*

**Abstract**

Endogenous arachidonic acid metabolites with properties similar to compounds of Cannabis sativa Linnaeus, the so-called endocannabinoids, have effects on various types of cancer. Although endocannabinoids and synthetic cannabinoids may have pro-proliferative effects, predominantly inhibitory effects on tumor growth, angiogenesis, migration and metastasis have been described. Remarkably, these effects may be selective for the cancer cells, while normal cells and tissues are spared. Such apparent tumor cell selectivity makes the endocannabinoid system an attractive potential target for cancer therapy. In this review we discuss various means by which the endocannabinoid system may be targeted in cancer and the current knowledge considering the regulation of the endocannabinoid system in malignancy.


*Keywords:* Endocannabinoid; Cannabinoid; Cancer; Lymphoma

## Contents



## 1. Discovering the potential use of the endocannabinoid system in cancer

The cannabinoid receptors and their endogenous ligands, the endocannabinoids, are expressed in mammalian tissue as well as in lower organisms such as invertebrates [1,2]. The highly conserved nature of the endocannabinoid system suggests that it constitutes an important biological regulatory system. Still, the cannabinoid receptors and their first identified endogenous ligands, arachidonoylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), remained un-discovered until recently [3–7]. In mammals, the endocannabinoid system has effects on many organ systems and it regulates cardiovascular, nervous, digestive, metabolic, reproductive and immune functions [8,9]. Studies to date indicate that the endocannabinoid system usually has suppressive effects, exemplified by decreased contractility in the heart, vasorelaxation, neuroprotection in acute and chronic neurological conditions

* Corresponding author. Tel.: +46 8 58581044; fax: +46 8 58581020.
*E-mail address:* birgitta.sander@ki.se (B. Sander).

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and anti-inflammatory effects. Both the $CB_1$ and the $CB_2$ genes encode seven-transmembrane G protein coupled receptors (reviewed in [10]). Initial studies suggested that $CB_1$ was expressed mainly in the central nervous system and $CB_2$ in the immune system. In soon became clear, however, that $CB_1$ receptors are expressed in most peripheral tissues including immune cells, albeit at lower levels than in CNS. Low levels of $CB_2$ have recently been detected in neuronal cells of the brain [11]. Putative other cannabinoid receptors have been suggested.

The natural plant cannabinoid, Δ-9-tetrahydrocannabinol (THC) was recognized as a potential anti-cancer agent already in 1975 [12]. However, it was not until the last 10–15 years that further studies in this area were carried out. Since then, there has been a great effort to investigate the therapeutic potential of cannabinoids in various types of cancer [13] and a first human clinical study has been performed [14]. Cannabinoids have been found to control cell growth and death in many cancer types but the mechanisms underlying the antitumor effects may vary and are sometimes cell type specific. Cannabinoids have been reported to cause heterogeneous effects in tumor cells, such as cell cycle alterations resulting in growth arrest, induction of apoptosis, anti-angiogenic activity and reduced migration. In some instances pro-proliferative effects of cannabinoids have been reported. Stimulation of cannabinoid receptors have been found to interfere with various intracellular signaling pathways and the efficacy of cannabinoids may be different in normal versus transformed cells.

## 2. The various components of the endocannabinoid system

The endocannabinoid system is commonly defined as the endocannabinoids together with their molecular targets and metabolizing enzymes.

In the early 1990s, the two seven-transmembrane G-protein coupled (GPCR) cannabinoid receptors, $CB_1$ [4,5] and $CB_2$ [7], were cloned. Based on their predominant expression in the central nervous system and in the peripheral immune system, $CB_1$ and $CB_2$ were initially denoted the central- and the peripheral cannabinoid receptor, respectively. It later became evident that $CB_1$ is also found at peripheral nerve terminals and in non-neuronal tissues, e.g. the pituitary gland, immune cells, vascular endothelium, eye, ileum and reproductive tissues [15,16], and that $CB_2$ is expressed also in the central nervous system [11]. However, the major role played by $CB_1$ is in inhibition of the release of neurotransmitters, whereas $CB_2$ mainly modulates functions of the immune system by regulating cell migration and cytokine release. Both cannabinoid receptors transmit signals via inhibition of adenylyl cyclase [17] and mitogen-activated protein kinases (MAPK) [18], whereas the neuromodulatory effect of $CB_1$ may be ascribed to inhibition of calcium channels [19] and activation of potassium channels [20]. In addition to their roles in neuromodulation and immune function, the cannabinoid receptors participate in the regulation of cell survival [21]. In this context, signaling via MAPK and phosphatidylinositol 3-kinase (PI3K)-AKT as well as generation of ceramide have been implicated [22].

The endocannabinoids were identified a few years after the cannabinoid receptors [23], the most well studied being the fatty acid amide AEA [3] and the monoacylglycerol 2-AG [6]. Both compounds are synthesized on demand from phospholipid precursors residing in the cell membrane in response to a rise in intracellular calcium levels, described in detail elsewhere [16]. Prior to its recognition as a ligand to cannabinoid receptors, 2-AG was known to act as an intermediate in various signaling pathways. 2-AG and AEA bind to $CB_1$ and $CB_2$ with similar affinities, but in a majority of investigations, 2-AG has been reported to display higher efficacy to $CB_1$ and $CB_2$ as compared to AEA [24]. Following release from the cells, AEA and 2-AG can act on molecular targets in an autocrine or paracrine manner, and are subsequently inactivated by cellular re-uptake. Different models of AEA transport including simple diffusion, endocytosis and transport mediated by a carrier protein have been discussed [25]. Properties of a putative AEA membrane transporter (AMT) have been described, although the molecular structure remains unknown [26].

Once inside the cell, the endocannabinoids are rapidly degraded. The major enzyme catalyzing the degradation of AEA is a fatty acid amide hydrolase (FAAH), which hydrolyzes AEA into arachidonic acid (AA) and ethanolamine (EA) (Fig. 1). 2-AG is converted into AA and glycerol by either FAAH or by the monoacylglycerol lipase MAGL (Fig. 1). Interestingly, biological activity of the degradation products has been reported. EA has been shown to protect neuroblastoma cells against apoptosis [27], and AA can be further metabolized to leukotrienes and prostaglandins with important roles in carcinogenesis, as reviewed in [28]. Other enzymes can, depending on the cellular context, contribute to the metabolism of AEA and 2-AG.

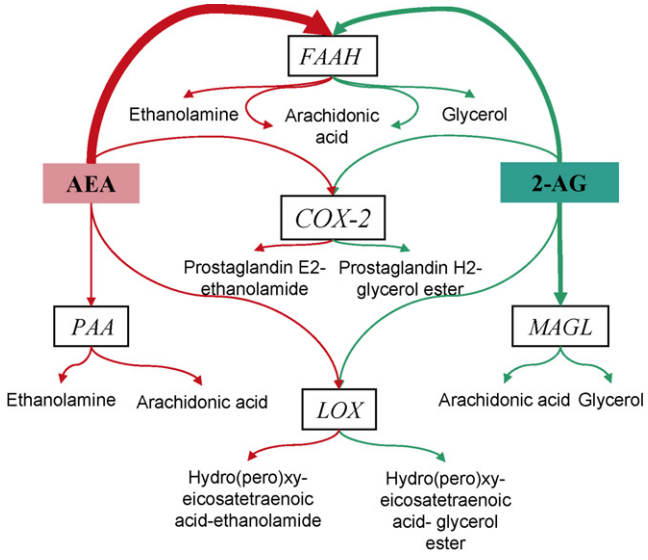


Fig. 1. Endocannabinoid metabolism. Fatty acid amide hydrolase (FAAH), cyclooxygenase-2 (COX-2) and lipoxygenases (LOXs) can use both *N*-arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG) as substrates, while palmitoylethanolamide-preferring acid amidase (PAA) and 2-arachidonoylglycerol monoacylglycerol lipase (MAGL) can metabolize AEA and 2-AG, respectively. As indicated by the width of the arrows, FAAH is the major enzyme degrading AEA, while FAAH and MAGL are of equal importance for the degradation of 2-AG.

While palmitoylethanolamide-preferring acid amidase (PAA) can process AEA, cyclooxygenase-2 (COX-2) and lipoxygenases (LOXs) can use both AEA and 2-AG as substrates (Fig. 1) [29]. The possibility has been raised that some of the metabolites generated by these enzymes could act as novel signal mediators.

In addition to cannabinoid receptors, the vanilloid receptor type 1 (transient receptor potential vanilloid 1, TRPV1) is well established as a target for AEA, while other receptors, e.g. peroxisome proliferator-activated receptor γ (PPARγ), the GPR55 orphan receptor and unidentified receptors have also been suggested [16,24]. AEA can also act as a modulator of other signalling pathways; allosteric sites for AEA are present on muscarinic and glutamate receptors.

Lipid mediators other than AEA and 2-AG, e.g. 2-arachidonoyl-ether (noladin ether), *O*-arachidonoyl-ethanolamine (virodhamine) and *N*-arachidonoyldopamine (NADA) have been reported to bind to $CB_1$ and $CB_2$, although the biological significance remains to be further investigated [30]. To add complexity, several endogenous, pharmacologically active fatty acid derivatives that do not bind to $CB_1$ and $CB_2$ can potentiate the activity of AEA and 2-AG, either by inhibiting the uptake or metabolism or by acting as substrates to the enzymes involved in transport or degradation [16].

## 3. Targeting the enzymes that inactivate endocannabinoids as a means to increase the endogenous tumor control

Endocannabinoids are produced by many cell types and their levels can be higher in cancer and pre-malignant conditions than in the surrounding normal tissue. The concentrations of the endocannabinoid AEA is approximately 75 nM in normal colon tissue and increases two- to three-fold upon malignant transformation [31]. In cell lines AEA and 2-AG levels are 40–200 nM [31], well in the range of activation of CB receptors [32]. However, cellular uptake of endocannabinoids is very rapid *in vivo* and *in vitro* [31]. Attempts have been made to increase the local concentration of endocannabinoids at the tumor cell surface by blocking endocannabinoid transport and the inactivating enzymes FAAH and MAGL. In this way anti-tumor effects of CB-receptor signaling have been induced in various cancer types such as thyroid, brain and prostate cancer [33–37]. In a xenograft model of thyroid cancer, substances that block endocannabinoid degradation increased levels of AEA and 2-AG in tissue and reduced tumor growth [33]. In prostate cancer cell lines, mainly effects on migration in vitro were reported [35–37]. These substances may however, have un-specific side-effects. In glioma and in lymphoma cells the AEA transport inhibitor VDM11 was found to have undesirable toxic effects *per se* [34] (Stranneheim and Flygare un-published observations). Blocking of endocannabinoid metabolism may also have other, un-expected effects as described by Matas et al. [27]. In neuroblastoma cells the AEA metabolite EA was protective against apoptosis induced by low serum. In this model the FAAH inhibitor URB597 induced cell death not by increasing AEA signaling through CB receptors but by preventing the generation of EA [27]. It is therefore likely that antiproliferative effects of endocannabinoid transport- and hydrolysis inhibitors could reflect combinations of direct toxic effects on the cells, of increased cannabinoid signaling and effects mediated by cannabinoid metabolites.

## 4. Expression of endocannabinoids and cannabinoid receptors in cancer

In a variety of human disorders, increased levels of endocannabinoids have been shown to reduce the severity of

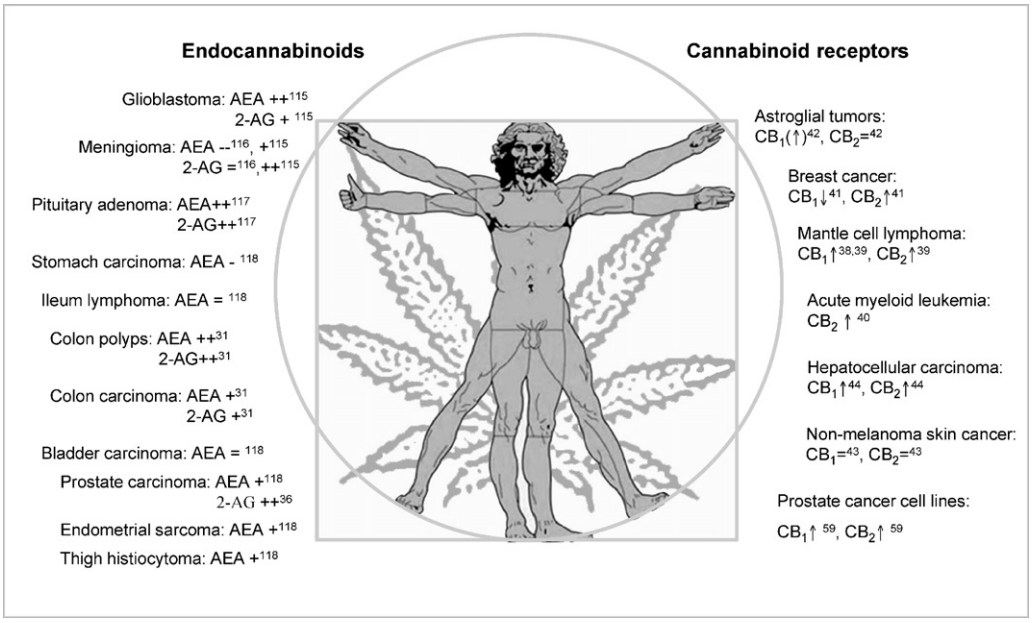


Fig. 2. Expression of endocannabinoids and cannabinoid receptors in cancer. Different cancer types showing altered expression of endocannabinoids or cannabinoid receptors as compared to the corresponding normal tissue = less than 1.5 × difference from control tissue, + or − more than 1.5 × difference from control tissue, ++ or −− more than 3× difference from control tissue, ↑ up-regulated and (↑) marginally up-regulated compared to control tissue.

symptoms or even oppose disease progression [8–10,16]. Elevated levels of endocannabinoids have been reported in several different types of cancer as compared to their normal counterparts (Fig. 2), whereas decreased or only marginally changed levels were observed in others. Intriguingly, the levels of both AEA and 2-AG were higher in precancerous polyps than in fully developed carcinoma in colon [31]. The authors suggest a role of the endocannabinoids as potential endogenous tumor growth inhibitors.

There are numerous reports on the effects of cannabinoids on cell survival and tumor growth [13,15,22], but only a few investigations on the expression of $CB_1$ and $CB_2$ compared to normal tissue (Fig. 2). While increased expression of $CB_1$ and/or $CB_2$ has been reported in mantle cell lymphoma [38,39], acute myeloid leukemia [40], breast cancer [41], hepatocellular carcinoma and prostate cancer cell lines, the levels of both receptors were similar to control levels in astroglial tumors [42] and non-melanoma skin cancer [43]. Interestingly, high expression of CB1 and CB2 was detected by *in situ* hybridization in cirrhotic liver samples and in well-differentiated human hepatocellular carcinoma, while the expression in poorly differentiated hepatocellular carcinoma was low [44]. The expression of cannabinoid receptors in liver thus follows the same pattern as the expression of AEA and 2-AG in colon, as discussed above, raising the possibility that an up-regulation of the endocannabinoid system at an early stage in cancer development might act as a host defense suppressing development of cancer.

## 5. Direct and indirect effects of cannabinoids on cancer: evidence for different mechanisms of action in different types of malignancies

Exogenous cannabinoids (natural from plants or synthetic analogues) may target tumor cells directly via binding CB-receptors thus affecting signaling and cellular pathways that eventually induce growth arrest, cell death or inhibit migration. They can also act indirectly, via inhibition of angiogenesis or interference with the immune system. Table 1 summarizes a selection of experiments performed *in vitro* that have elucidated various mechanisms of action in different cancers. Since the efficacy of cannabinoids *in vitro* is dependent on the concentration of serum in the culture medium (usually with less potency at higher serum concentrations [45]) this variable is included in the table in order to facilitate comparison between different studies. The low serum concentrations used in some of the experiments may seem artificial, but can in fact mimic conditions in interstitial compartments in tumors where extracellular macromolecules can restrict the distribution of serum proteins [46]. Low serum concentrations may also induce oxidative stress in cells, thereby increasing the sensitivity to cannabinoids [47,48].

In brain tumors (mainly glioma including the highly malignant variant glioblastoma multiforme) cannabinoids induce cell death by stimulating ceramide synthesis de novo, resulting in a sustained ceramide accumulation, long-term up-regulation of ERK peaking on days 3–5 and apoptosis [49]. Antagonistic effects on growth factor induced proliferation has also been reported [42]. Usually, the effects are tumor selective, sparing normal cells [50,51]. The effects have been further investigated using xenograft models where cannabinoids lead to growth inhibition of transplanted tumors [52–55]. A pilot clinical study of patients with glioblastoma multiforme has evaluated the safety of intracranial THC administration. Importantly, these tumors expressed CB receptors and remained sensitive to the pro-apoptotic effect of THC also after *in vivo* treatment [14].

Breast cancer may be dependent on hormones, such as estrogen and prolactin, for growth and survival. Many cultured breast cancer cell lines express prolactin receptors and are dependent on autocrine prolactin for pro-proliferative stimulation. In such cell-lines cannabinoids mainly induce growth arrest by down-regulating prolactin receptors [56]. Also pro-proliferative effects of exogenous prolactin was counteracted. Thus, breast-cancer proliferation depends on an autocrine prolactin loop and signaling through $CB_1$ has been shown to down-regulate the prolactin receptor and indirectly inhibit cell growth. Interestingly, these effects were also seen when FAAH was blocked, indicating that the cells produced endocannabinoids that could inhibit proliferation if present in adequate amount [56]. In further studies, anti-proliferative effects of cannabinoids mediated by down regulation of the high affinity NGF receptor Trk was demonstrated [57]. Cannabinoids have also been shown to inhibit breast cancer *in vivo* via acting on $CB_1$ [58].

Prostate cancer cell lines express higher levels of $CB_1$ and $CB_2$ than benign prostate epithelium [59]. Similarly to breast cancer, prostate cancer may express prolactin receptors and proliferate in response to prolactin and this response can be inhibited by cannabinoids [57]. Also the EGFR expression level can be down-modulated by cannabinoids, resulting in a $CB_1$ mediated inhibition of proliferation at day 3 and in massive cell death by apoptosis/necrosis at day 5, mediated through $CB_1$ and $CB_2$ [60]. Cannabinoids have also been reported to down-regulate the androgen receptor and prostate specific antigen (PSA) [61]. However, the sensitivity to cannabinoids seems to be quite variable in different prostate cancer cell lines even when they express CB-receptors [60,61,48].

Normal and transformed colon mucosa contain $CB_1$, $CB_2$ and FAAH [31] and most colon cancer cell lines also express both CB-receptors [62]. In a recent study it was reported that THC induce apoptosis by a $CB_1$ mediated effect, via dephosphorylation of ERK and AKT and activation of BAD [62], similar to findings in glioma [63] and in T cell lymphoma and leukemia [64]. However, the sensitivity to $CB_1$ versus $CB_2$ ligands might differ among colon cancer cell lines and during differentiation [31]. These tissues and cell lines also produce the endocannabinoids AEA and 2-AG, which are increased two- to three-folds in malignant tissue compared to normal [31]. Upon blockage of endocannabinoid inactivation the levels of AEA and 2-AG increased and the cell proliferation decreased. It was therefore suggested that endocannabinoids act as endogenous tumor growth inhibitors [31].

The $CB_2$ receptor was cloned from the human pro-myelocytic leukemia HL-60 [7]. Shortly thereafter it was found that a common retroviral integration site was located near the Cb2 locus in mice [65]. In spite of these highly interesting reports there is still very limited knowledge on the effects of cannabi-

Table 1
Effects of cannabinoids on cancer cells during *in vitro* culture

| Cancer[a] | Agent | FCS | Effect in vitro | Receptor | Cycle arrest | Apopt | Growth factor | Angio | ER stress | Oxid stress | $Ca^{2+}$ | PTX | cAMP/ PKA | PI3/ AKT | PKB | ERK | p38 MAPK | cer | COX-2 | LOX | necr | Rafts | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Brain | THC16 | Low | AA release, PGE2-prod | ND | | | | | | | | | | | | | | | | | | | [119] |
| | THC1 | 0 | Apoptosis, sphingomyelin hydrol | Non-$CB_1$ | | 1 | | | | | | | | | | | | 1 | | | | | [120] |
| | AEA10 | 0 | apoptosis | TRPV1# | | 1 | | | | | 1* | | | | | | | | 1* | 1* | | | [93] |
| | THC 1; WIN 0.02; HU210 0.01; CP55,940 0.45 | 0 | Cell death | $CB_1$, $CB_2$ | | 1 | | | | | | | 0 | | | 1 | 0 | 1 | | | | | [54] |
| | THC1; CBD1; AEA1n.e.; MAEA1n.e. | 0 | Reduced viability | ND | | | | | | | | | | | | | | | | | | | [45] |
| | AEA2; 2-AG2; WIN10 | Low | Anti-proliferative | $CB_1$, $CB_2$, TRPV1 | | | | | | 1 | | | | | | | | | | | | | [47] |
| | THC10; MAEA10 | 0 | induction of COX2 | Non-CB | | | | | | | | 0 | | | | 1 | 1 | | | | | | [121] |
| | JWH133 0.1 | 0 | reduced viability | $CB_2$ | | 1 | | | | | | | | | | 1 | | 1 | | | | | [55] |
| | THC 0.5 | 0 | apoptosis | ND | | 1 | | | | | | | | | 1 | 1 | | 1 | | | | | [49] |
| | AEA 10; MAEA 10; WIN 10; HU210 10 | 0 | Induction of COX-2 | Non-CB non TRPV1 | | | | | | | | | | | | 1 | 1 | 1 | | | | | [122] |
| | AEA3; 2-AG3; MAEA 10 | Low | Anti-proliferative | $CB_1$, $CB_2$, TRPV1 | | | | | | | | | | | | | | | | | | | [123] |
| | THC 1; AEA 10; WIN 10; HU210 0.05 | 0 | Pro-survival via EGFR | ND | | | EGFR | | | | | | | 1 | 1 | 1 | | | | | | | [80] |
| | AEA 2; WIN 0.1; JWH133 0.1 | Low | Reduc pVEGFR-2, reduc VEGF-prod | $CB_1$, $CB_2$ | | | | 1 | | | | | | | | | | 1 | | | | | [53] |
| | THC 2; WIN 1.25 | Low | Anti-proliferative | $CB_1$, $CB_2$ | | | | | | | | | | | | | | | | | | | [51] |
| | THC 1.6 | 0 | Reduced viability | $CB_1$, $CB_2$ | | 1 | | | 1 | | | | | | | | | 1 | | | | | [85] |
| | Anandamide transport inhib. | Low | Cell death | Non-CB | | | | | | 1 | | | | | | | | | | | | | [34] |
| | THC 2.5 | Low | Reduced viability | $CB_1$, $CB_2$ | | | | | | | | | | | | | | | | | | | [14] |
| | WIN 0.05; JWH133 0.016; HU210 0.0003 | Low | Anti-proliferative, antagonise bFGF | ND | | 0 | | | | | | | | | | | | | | | | | [42] |
| | THC 14 → 25; CBD 6-20 | N | Anti-proliferative, pro-apoptotic | Variabl. | | | | | | 1CBD | 1CBD | | | | | | | | | | | 0CBD | [48] |
| | AEA 0.5–5, hydrolysis inhib. | Low | Anti-apoptic in low serum | FAAH | | 1 | | | | | | | | | | | | | | | | | [27] |
| | HU-210 0.03; JWH-133 0.03 | 0 | Differentiation of glioma stem cells | $CB_1$, $CB_2$ | | | | | | | | | | | | | | | | | | | [109] |
| Breast | AEA5; 2-AG1; MAEA 5; HU2105 | N | Anti-proliferative | $CB_1$ | 1 | 0 | prolac | | | | | | | | | | | | | | | | [56] |
| | AEA2,5; HU210 2.5 | 0 | Anti-proliferative | $CB_1$ | | | prolac, NGF | | | | | | 1 | | | 1 | | | | | | | [124] |
| | AEA 1; MAEA 1; HU210 1 | 0 | Suppress NGF rec, anti-proliferative | $CB_1$ | | | prolac, NGF | | | | | | | | | | | | | | | | [57] |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AEA0.04; JWH133 0.01 | N | inhibition of migration | ND | | | | | | | | | | | | | | | | | | | | | | | | | | | [125] |
| | MAEA 10 | N | Anti-proliferative, anti-migratatory | $CB_1$ | | 0 | | | | | | | | | | | | | | | | | | | | | | 0 | | | [58] |
| | THC 20 | N | n.e. on cancer cell viability in vitro | Immun sup-press | | | | | | | | | | | | | | | | | | | | | | | | | | | [87] |
| | THC 5 | Low | Anti-proliferative, pro-apoptotic | $CB_2$ | 1 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | [41] |
| | THC 14 → 25 | N | Anti-proliferative, pro-apoptotic | Variabl. | | | | | | | 1CBD | 1CBD | | | | | | | | | | | | | | | | | 0CBD | | [48] |
| | SR141716A1 | N | anti-proliferative | $CB_1$ | 1 | 0 | | | | | | | | | | | 1 | | | | | | | | | | | 0 | 1 | | [92] |
| | MAEA 10 | N | Anti-proliferative | ND | 1 | 0 | | | | | | | | | | | | | | | | | | | | | | | | | [126] |
| Thyroid | MAEA 10 | N | Anti-proliferative | $CB_1$ | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | [83] |
| | 2-AG 10; MAEA 5; transport/hydrolysis inhib | N | Anti-proliferative | $CB_1$ | | 0 | | | | | | | | | | | | | | | | | | | | | | | | | [33] |
| | MAEA 10 | N | Anti-proliferative | $CB_1$ | 1 | 0 | | | 1 | | | | | | | | | | | | | | | | | | | | | | [76] |
| | THC 14 → 25; CBD 6-20 | N | Anti-proliferative, pro-apoptotic | Variabl. | | | | | | | 1CBD | 1CBD | | | | | | | | | | | | | | | | | 0CBD | | [48] |
| Pancreas | THC 2 | 0 | Reduced viability | $CB_2$ | | 1 | | | | 1 | | | | | | | | | | 1 | | | | | | | | | | | [84] |
| | THC 1.6 | 0 | Reduced viability | $CB_1$, $CB_2$ | | 1 | | | | 1 | | | | | | | | | | 1 | | | | | | | | | | | [85] |
| Prostate | THC1 | 0 | Cell death | Non-CB | | 1 | | | | | | | | 0 | | | | | | | | | | | | | | | | | [127] |
| | AEA 1; MAEA 1; HU210 1 | 0 | Suppress NGF rec, anti-proliferative | $CB_1$ | | | prolac, NGF | | | | | | | | | | | | | | | | | | | | | | | | [57] |
| | THC 0.1 | 0 | Induce NGF production | $CB_1$ | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | [128] |
| | AEA 2 | 0 | Anti-proliferative, pro-apoptic | $CB_1$, $CB_2$ | 1 | Day 5 | EGFR d.3 | | | | | | | 1 | d.3 | | | | | d.5 | | | | | | | | | | | [60] |
| | 2-AG 1; MAEA 1; WIN 0.1; hydrolysis inhib | 0 | Inhib migration | $CB_1$ | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | [36] |
| | hydrolysis inhib | 0 | Inhib migration by enhancing 2-AG | $CB_1$ | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | [37] |
| | WIN 5 | N | Reduced viability | $CB_1$, $CB_2$ | | 1 | androgen rec | 1 | | | | | | | | | | | | | | | | | | | | 1 hi | | | [59] |
| | WIN 7.5–20 | N | Cell death | $CB_1 + CB_2$l | | 1 | | | | | | | | | | 1 | | 1 | | | | | | | | | | | | | [61] |
| | THC 14 → 25; CBD 6–20 | N | Anti-proliferative, pro-apoptotic | Variabl. | | | | | | | 1CBD | 1CBD | | | | | | | | | | | | | | | | | 0CBD | | [48] |
| | 2-AG 10; hydrolysis inhib | 0 | Pro-migration by inducing 12-LO | ND | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | [35] |
| Gyn | AEA 30 | N | Cell death | TRPV1# | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | [94] |
| Lung | THC 10 | 0 | Anti-proliferative | ND | | | | | | | | | | | | | | | | | | | | | | | | | | | [12] |
| | MAEA 14 | N | Pro-prol | Non-CB | | | | | | | | | | | | | | | 1 | | 1 | | | | | | | | | | [96] |
| | MAEA 10 | N | Anti-prol | $CB_1$ | 1 | 0 | | | 1 | | | | | | | | | | | | | | | | | | | | | | [76] |
| | THC1; AEA10; WIN10; HU210 0.05 | 0 | Pro-survival via EGFR | ND | | | EGFR | | | | | | | | | 1 | | 1 | | 1 | | | | | | | | | | | [80] |
| Skin | WIN 0.025; JWH133 0.025 | Low | Reduced viability | $CB_1$, $CB_2$ | | 1 | EGFR | 1 | | | | | | | | | | | | | | | | | | | | | | | [43] |
| | THC 1.6 | 0 | Reduced viability | $CB_1$, $CB_2$ | | 1 | | | | 1 | | | | | | | | | | 1 | | | | | | | | | | | [85] |

Table 1 (*Continued*)

| Cancer[a] | Agent | FCS | Effect in vitro | Receptor | Cycle arrest | Apopt | Growth factor | Angio | ER stress | Oxid stress | $Ca^{2+}$ | PTX | cAMP/ PKA | PI3/ AKT | PKB | ERK | p38 MAPK | cer | COX-2 | LOX | necr | Rafts | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | THC 1; WIN 0.1 | Low | Anti-proliferative, pro-apoptic | $CB_1$, $CB_2$ | 1 | 1 | | | | | | | | 1 | | | | | | | | | [52] |
| Gastroint. | AEA 2.5; 2-AG 2.5; HU2101 | N | Anti-proliferative | $CB_1$, $CB_2$ | | | | | | | | | | | | | | | | | | | [31] |
| | AEA 0.04; JWH133 0.01 | 0 | Inhibition of migration | ND | | | | | | | | | | | | | | | | | | | [125] |
| | AEA 25, hydrolysis inhib | Low | Cell death | ND | | 0 | | | | | | | | | | | | | 1 | | 0 | | [129] |
| | AEA 0.1; MAEA 0.01; WIN 0.1; JWH 0.02 | 0 | Stim. Of migration on plastic | $CB_1$ | | | | | | | | 1 | | 1 | 1 | 1 | | | | | | | [130] |
| | THC 14 → 25; CBD 6–20 | N | Anti-proliferative, pro-apoptotic | Variabl. | | | | | | 1CBD | 1CBD | | | | | | | | | | | 0CBD | [48] |
| | THC 10 | 0 | Apoptosis | $CB_1$ | | 1 | | | | | | | | 1 | | 1 | | | | | | | [62] |
| | AEA10; 2-AG10n.e. | ND | Cell death by AEA (prolif by 2-AG) | Non-CB | | 1 | | | | | | 0 | | | | | | 1 | | | | 1 | [95] |
| Hemato | THC 10 | N | Differentiation | ND | | | | | | | | | | | | | | | | | | | [131] |
| | AEA 10 | 0 | Pro-proliferative in synergy with GF | ND | | | | | | | | | | | | | | | | | | | [81] |
| | AEA 0.5; MAEA 0.5 | 0 | Pro-proliferative in synergy with GF | Non-CB | | | | | | | | 0 | | | | 1 | | | | | | | [82] |
| | AEA 10 | 0 | Apoptosis | TRPV1# | | 1 | | | | | 1* | | | | | | | | 1* | | | | [93] |
| | CP55,940 0.01–0.1 | Low | Cytokine production, migration | $CB_2$ | | | | | | | | | | | | 1 | | | | | | | [66] |
| | THC10; HU210 5 | 0 | apoptosis | $CB_2$ | | | | | | | | | | | | | | | | | | | [75] |
| | 2-AG 0.3 | N | differentiation block | $CB_2$ | | | | | | | | | 0 | 1 | | 1 | 0 | | | | | | [132] |
| | AEA 5; WIN 5 | Low | Growth inhibition, reduced viability | ND | | 1 | | | | | | | | | | | | | | | | | [71] |
| | THC1.5; JWH13310 | 0 | Apoptosis | $CB_2$ | | | | | | | | | | | | 0 | 1 | | | | | | [74] |
| | MAEA 10; WIN 10 | 0 | Apoptosis | $CB_1$, $CB_2$ | | | | | | | | | | | | (1) | 1 | 1 | | | | | [72] |
| | THC 1.5 | 0 | Apoptosis | $CB_2$ | | | | | | | | | | | | | | 1 | | | | | [73] |
| | THC 10 | N | Apoptosis | $CB_1$, $CB_2$ | | | | | | | | 1 | | 0 | | 1 | 0 | | | | | | [64] |
| | THC 14 → 25; CBD 6–20 | N | Anti-prol, pro-apoptotic | Variabl. | | | | | | 1CBD | 1CBD | | | | | | | | | | | 0CBD | [48] |

[a] Abbreviations: cancer: cancer type; agent: cannabinoid concentration in μM; FCS: % fetal calf serum in culture medium, 0 = serum free, low ≤ 5%, *N* = 5%and higher; cycle arrest: cell cycle arrest; apopt: apoptosis; angio: angiogenesis; ER: endoplasmatic reticulum; oxid: oxidative; PTX: pertussis toxin; cer: ceramide; necr: necrosis; rafts: lipid rafts; ref: reference; THC: tetrahydrocannabinol; AA: arachidonic acid; PGE2: prostaglandin E2; WIN: WIN-55,212-2; MAEA: methanandamide; n.e.: no effect; CBD: cannabidiol; EGFR: epidermal growth factor receptor; pVEGFR: phosphorylated vascular endothelial growth factor receptor; prolac: prolactin; bFGF: basic fibroblast growth factor; NGF: nerve growth factor. Other symbols: # protective effect of CB-receptors; * cells without CB-receptors; 1 hi: at high dose.

noids in myeloid malignancies such as acute myeloid leukemia (AML), myelodysplastic syndromes (MDS) or myeloproliferative disorders. It has been reported that approximately 50% of primary AML express $CB_2$ [40]. There are also reported effects of cannabinoids on cell migration and production of cytokines from myeloid cell lines [66]. In contrast, the initial findings of $CB_1$ and $CB_2$ on lymphocytes and other immune cells [67–69] stimulated extensive studies on the importance of the endocannabinoid system in immunity (reviewed in [70]) and also in lymphoid malignancies. Gene expression profiling of a malignant lymphoma arising from B lymphocytes, mantle cell lymphoma (MCL), demonstrated high expression of $CB_1$ [38,39] and $CB_2$ [39] in the tumor compared to reactive lymphoid tissue. Targeting of the CB-receptors by agonists induced cell death in MCL tumor cell lines and primary MCL cells [71] while normal B lymphocytes from tonsil or peripheral blood were spared [72]. Both $CB_1$ and $CB_2$ induced p38 MAPK activation, de novo ceramide synthesis and participated in the signaling pathway to apoptosis [72]. However, in lymphoid malignancies emanating from T lymphocytes, such as T lymphoblastic leukemia, cannabinoids can exert their effects by targeting $CB_2$ only [73–75]. THC induces $CB_2$ dependent p38 MAPK activation and apoptosis in the T cell leukemia line Jurkat at 2 h [73]. However, at other culture conditions, THC induced up-regulation of $CB_1$ receptor mRNA in Jurkat cells. THC could then, via $CB_1$ and $CB_2$, inhibit ERK-signaling, induce translocation of Bad to mitochondria and cause apoptosis at 12 h [64].

Tumors depend on the formation of new vessels from pre-existing vasculature (angiogenesis) to grow beyond a size of a few millimeters. Many cancers can therefore produce pro-angiogenic factors such as vascular endothelial growth factor, VEGF, in response to hypoxia. In addition to their direct growth suppressing and pro-apoptotic effects on tumor cells, cannabinoids can inhibit angiogenesis via effects on production of pro-angiogenic factors by cancer cells or by a direct effect on vessel formation in vivo. This has been especially well studied in glioma, skin cancer and thyroid cancer. Production of VEGF from these cell lines is inhibited by cannabinoids [53,76]. In addition, cannabinoids can directly target the tumor vasculature. Primary endothelial cells express $CB_1$ and $CB_2$ [77–79] and are highly sensitive to apoptotic induction by cannabinoids at low nM doses [77]. Cannabinoids also inhibit bFGF induced endothelial cell proliferation, tube formation and sprouting and induces apoptosis in endothelium *in vitro* and *in vivo* by acting on $CB_1$ [79]. Experiments *in vivo*, using xenotransplanted glioma show that vessels have different morphology and permeability after treatment with a selective $CB_2$ agonist [77]. In a pilot clinical study, diminished production of VEGF and reduced activation/expression of VEGF-2 receptor was reported in patients receiving intracranial THC as part of the previously mentioned clinical study on glioblastoma multiforme [53].

## 6. Growth promoting effects by cannabinoids/endocannabinoids in cancer

When exploring the therapeutic potential of cannabinoids in cancer, also the possible tumor stimulating effects have to be considered. In fact there are a few reports suggesting that cannabinoids may have pro-proliferative effects in cancer. In various cancer cell lines, representing lung cancer, squamous cell carcinoma, bladder carcinoma, glioblastoma, astocytoma and kidney cancer, cannabinoids such as THC, AEA and WIN induced transactivation of the EGF-receptor by metalloprotease mediated cleavage of growth factor precursors [80]. These effects were seen at 1/10 of the pro-apoptotic concentration, i.e. at sub-μM doses of THC, concentrations that may occur during intermittent treatment with a drug [80]. In various hematopoietic and lymphoid cell lines, dependent on growth factors such as IL-3 or IL-6, sub-μM doses of AEA [81,82], MAEA, and free AA potentiated growth factor induced proliferation at low serum conditions. This effect was not mediated via cannabinoid receptors but involved a $CB_1$/$CB_2$ independent stimulation of p42/p44 MAPK [82]. However, in other lymphoid cell lines or in primary lymphoma of the mantle cell type cannabinoids did not promote spontaneous [71] or CD40 mediated proliferation (Flygare, Eriksson and Sander unpublished observations).

Since cannabinoids can suppress cell mediated and humoral immune responses [70], effects on tumor immunity have to be taken into account when considering targeting the cannabinoid system *in vivo*. If tumors express CB-receptors, both tumors and immune cells are targeted by receptor agonists with inhibitory effects on tumor growth and metastasis in xenograft models ([12,33,43,48,50,52–55,58,75–77,79,83–85]. Similar mechanisms operate in inflammatory disorders [86]. If, however, the cancer cells do not express CB-receptors, they have been shown to be relatively resistant to the effects of cannabinoids [87]. Administration of cannabinoids, such as THC, to mice transplanted with cancers lacking CB-receptors may promote *in vivo* cancer growth through suppression of the anti-tumor immune response [87].

## 7. Anti-cancer effects of the $CB_1$ antagonist SR141716A

The $CB_1$ receptor ligand SR141716A was introduced in the mid-1990s [88] and its potent and selective $CB_1$ receptor antagonist activity has been of tremendous value in cannabinoid research. In some experiments it has been noted that SR141716A not only has antagonistic capacities and inhibit the effects of $CB_1$ agonists, but in fact has effects *opposite* of those produced by agonists. One explanation for such phenomena could be that the $CB_1$ receptor has a low, basal signaling capacity even in the absence of agonist and that this effect is blocked by SR141716A that thus behaves as an inverse agonist (reviewed in [89]). SR141617A (Rimonabant/Acomplia) is now used as treatment for obesity and the metabolic syndrome. In experimental settings SR141716A has a direct effect on adipose tissue and reduces proliferation of adipocytes via inhibiting p42/44 MAPK activity [90].

In various cancers SR141716A in the nM–low μM range reverts the effect of CB-receptor agonists in *vivo* and *in vitro*. These potent antagonizing effects may cause concern whether treatment with CB-receptor antagonists in some instances could enhance cancer proliferation (reviewed in [91]). However, such effects have not been described. When used on various cancer

cell lines in vitro, SR141716A either antagonized the anti-cancer effects of cannabinoids or had no reported stimulatory effect on cell proliferation or viability. In fact, significant growth inhibiting effects of μM doses of SR141716A on breast cancer, colon carcinoma and thyroid cancer cell lines [56,33] and on primary cells and cell-lines from malignant lymphoma have been reported [71]. The anti-tumor effects of low doses of SR141716A in breast cancer was mediated through $CB_1$ and was associated with inhibition of signaling through ERK, down-regulation of cyclin D1 and cell cycle arrest [92]. Importantly, SR141716A has also been reported to reduce tumor growth in xenograft models [92]. Together, these findings provide evidence that $CB_1$ antagonists may have a complex mechanism of action that can be difficult to predict, even when based on well performed earlier experimental studies.

## 8. Effects independent of cannabinoid receptors in cancer

In addition to their CB-receptor mediated effects, the endocannabinoids, and in particular AEA, can mediate various CB-receptor independent signaling effects via the TRPV1 receptor [93,94,100] as discussed above. In fact, signaling through cannabinoid receptors have in some instances been found to protect cancer cells against TRPV1 mediated cell death [93,94]. It has also been suggested that AEA induce receptor-independent cell death by interacting with cholesterol rich membrane domains, so-called lipid rafts. This was the case in cholangiocarcinoma where AEA inhibited proliferation and induced apoptosis by a $CB_1$/$CB_2$/TRPV1 independent pathway involving lipid rafts, ceramide accumulation and activation of death receptor pathways [95]. As discussed earlier, AEA and other cannabinoids may interact with the COX and LOX pathways. This may explain some apparently conflicting results, such as in animal models of lung cancer where MAEA has been reported both to promote and inhibit metastasis [76,96]. In one of these studies MAEA stimulated cancer cell proliferation in a $CB_1$/ $CB_2$ receptor independent manner *in vitro* and *in vivo* via induction of COX-2 and increased production of prostaglandins [96]. However, a 10-fold lower dose of MAEA in the same syngenic lung cancer model instead reduced the frequency of lung metastases in a $CB_1$ dependent way [76].

## 9. Selective effect of cannabinoids in cancer cells while normal cells are spared

As discussed above, there is growing evidence that cannabinoids may selectively target tumor cells while normal cells are less sensitive. A few possible underlying mechanisms for this desired effect have been suggested recently. In some instances there might be a differential use of signaling pathways in transformed cells. One signaling pathway that has been reported to be differentially regulated by cannabinoids in normal cells versus cancer is the RAS-MAPK/ERK pathway in brain cells [50]. The responses of glioma cells and astrocytes exposed to THC are completely different. THC induces ceramide synthesis and cell death in glioma cells but not in astrocytes which are instead protected from oxidative stress by cannabinoids in a $CB_1$-mediated manner [97]. In other cancers the selective response to cannabinoids might reflect qualitative or quantitative differences in expression of cannabinoid receptors (Fig. 2) [38,39,41,52,59,72,83,84] or FAAH [98]. In malignancies, such as thyroid cancer, lymphoma, melanoma, pancreas and breast cancer, the levels of cannabinoid receptors are often higher in the tumor compared to normal cells of the same origin (Fig. 2), resulting in increased sensitivity to cannabinoids in the malignancies [38,39,41,52,59,72,83,84]. Similarly, anti-proliferative and pro-apoptotic effects of cannabinoids on tumor cells and not on normal tissue have been reported in animal studies [54,84].

There are also interesting experiments suggesting that malignant cells may respond differently to cannabinoids depending on their state of differentiation. The colon cancer cell line $CaCo_2$ starts to differentiate when reaching confluence. In undifferentiated cells cannabinoids were strongly anti-proliferative via acting on $CB_1$. However, there was no effect on proliferation in differentiated cells. Intriguingly, while the overall levels of $CB_1$ did not change after differentiation, an alteration in $CB_1$ glycosylation was suggested and possibly affected cell signaling [31].

## 10. Regulation of cannabinoid receptor expression in cancer

The different response of normal and malignant cells to cannabinoids and the abnormal expression of CB-receptors in cancer compared to normal tissue (Fig. 2) calls for further research on the regulation of cannabinoid receptors. This is also of importance when considering treating primary cancers since many (but not all, as discussed above) of the effects of cannabinoids are mediated through CB-receptors. There are a few studies that investigate how expression of CB-receptors may vary within well-defined cancer subtypes. It has been difficult to find really good and specific antibodies for immunohistochemical staining and therefore some assays of tissue expression levels have been made by PCR-methods or immunoblot, which give limited information concerning cell types that express the receptors *in vivo*. Still a very interesting picture has emerged. Analyses of $CB_2$ expression in astrocytoma by PCR demonstrated that 70% of tumors expressed $CB_1$ and/or $CB_2$ [55]. In another study most of the brain tumors expressed $CB_1$ and 50% of tumors expressed $CB_2$ mRNA [42]. Similar finding were reported when assaying $CB_2$ levels in adult and pediatric brain tumors [99]. In primary breast cancer $CB_1$ mRNA levels varied by a factor of 30 and many breast cancer expressed lower levels of $CB_1$ than normal breast tissue. In contrast most breast cancers hade higher expression of $CB_2$ mRNA than normal breast [41]. Also in pancreatic cancer there seemed to be a large variation in expression of CB-receptors among primary cancers [84] while there was less variation in uterine cervical cancer [94]. From these studies it can be concluded that while a majority of cancers express CB-receptors, a substantial fraction of tumor tissue seem to express very low receptor levels. The findings are still more intriguing if the mixed composition of tumor tissue is taken into account and it could be hypothesized that

the tumor microenvironment may regulate CB-receptor expression. Is there any evidence in support of such a hypothesis? The promoter regions of the $CB_1$ receptor in mouse and man have recently been described [101,102]. At present there is however only limited knowledge on the regulation of CB-receptor expression in tumor tissue. Some knowledge can instead be gained from the situation in normal cells: in neuronal cells, expressing cannabinoid receptors, exposure to agonists may lead to pharmacological tolerance, desensitization and reduced expression of the receptor at the cell surface [103]. In order to express new receptors, protein synthesis is required [103,104]. Other cell types might react differently and instead up-regulate CB-receptors after agonist stimulation [83,105]. In peripheral blood lymphocytes, higher levels of $CB_1$ and $CB_2$ mRNA were seen in response to chronic exposure to cannabinoids [106]. Normal, unstimulated T lymphocytes have been reported to express only a few hundred $CB_2$ receptors [107]. However, very few receptors might be sufficient to produce significant changes in cellular regulation, since primary T cells are responsive to sub-μM doses of THC or $CB_2$ specific agonists [105]. Interestingly, in primary T lymphocytes and the T cell lymphoma lines Jurkat and CEM, stimulation of $CB_2$ receptors by cannabinoids induced a 10–400-fold increase in $CB_1$ mRNA expression [105] via a pathway that involved STAT5 activation and induction of IL-4. IL-4 in turn induced $CB_1$ expression via activation of STAT6 [105]. In immune cells, CB-receptors may also be modulated during stimulation with polyclonal activators or antigen. Also in epithelial cells, such as normal colon epithelium and in the colon cancer cell line HT29, inflammatory stimuli or bacteria can upregulate $CB_1$ [108]. This suggests that the tumor environment can indeed alter the CB-receptor phenotype in cancer.

A different picture has been described in malignant glioma. Cells with self-renewing capacity, so-called glioma stem cells (tumor stem cells) can be isolated from tumor biopsies and grown *in vitro*, forming three-dimensional aggregates of cells called neurospheres. The cells in these neurospheres expressed CB-receptors and exposure to cannabinoids induced expression of markers characterizing a more differentiated cell stage. Importantly, cannabinoids did not impair the proliferation or the self-renewing capacity of the glioma derived stem cells *in vitro*. However, when the cannabinoid-exposed glioma stem cells were transplanted to animals they exhibited less tumor forming capacity compared to the un-exposed counterparts [109]. These observations give rise to at least two important questions that can be addressed in further experiments: Are "tumor stem cells" in general less sensitive to the anti-proliferative effects of cannabinoids? Are cells that have differentiated in response to cannabinoids still sensitive to the cannabinoid action?

There are yet other levels of regulation of the expression of $CB_1$. This receptor protein has an unusually long amino-terminal tail that impairs the translocation of the receptor over the ER membrane, resulting in low receptor levels at the cell membrane [110]. However, truncation of part of the amino-terminal has been reported to occur if the receptor is expressed in certain cells. The truncation results in high membrane expression of a truncated form of $CB_1$ that may have novel features compared to the native $CB_1$ [111]. It is also clear that different splice variants of $CB_1$ exist, at least in the brain [112]. Interestingly, some of these splice variants display different binding and signaling efficacies. This certainly adds to the complexity of the field and will most likely be the focus of interesting future studies.

## 11. Targeting the endocannabinoid system in cancer—where are we today?

In the now classical review by Hanahan and Weinberg [113] six essential alterations in cell physiology that dictate malignant growth were listed as hallmarks for cancer development; self-sufficiency in growth signals, insensitivity to anti-growth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis. The emerging picture of the role of the endocannabinoid system in cancer indicates that this system is involved in regulating many of these functions that are essential in cancer development. Numerous studies have shown that interference with the endocannabinoid system can inhibit cell growth, induce apoptosis, have effects on cancer stem cells, impair angiogenesis and reduce tissue invasion and metastasis (Table 1). This knowledge has also spurred a clinical trial examining the safety and efficacy of giving THC locally in malignant glioma [14]. However, successful cancer treatment is most often obtained by using combinations of therapies such as surgery, chemotherapy, antihormonal treatment and targeted therapy using antibodies or small molecules such as signal transduction inhibitors. Future studies will clarify if targeting of the endocannabinoid system may be added to this therapeutic arsenal. Apart from the effects discussed above there are additional aspects of the endocannabinoid system that are promising in this respect. Signaling through CB-receptors is coupled to the generation of ceramide, a molecule that is involved in the regulation of cancer growth by mediating anti-proliferative and pro-apoptotic effects. Many anti-cancer drugs also induce cell death by stimulating the generation of ceramide. Attenuation of ceramide levels is a feature of cancer pathogenesis, usually coupled to resistance to apoptosis (reviewed in [114]). Future research should certainly address the question of whether combining chemotherapeutic substances and cannabinoids may have synergistic effects as anti-cancer agents.

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