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Update on the endocannabinoid system as an anticancer target

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Introduction: Recent studies have shown that the endocannabinoid system (ECS) could offer an attractive antitumor target. Numerous findings suggest the involvement of this system (constituted mainly by cannabinoid receptors, endogenous compounds and the enzymes for their synthesis and degradation) in cancer cell growth *in vitro* and *in vivo*.
Areas covered: This review covers literature from the past decade which highlights the potential of targeting the ECS for cancer treatment. In particular, the levels of endocannabinoids and the expression of their receptors in several types of cancer are discussed, along with the signaling pathways involved in the endocannabinoid antitumor effects. Furthermore, the beneficial and adverse effects of old and novel compounds in clinical use are discussed.
Expert opinion: One direction that should be pursued in antitumor therapy is to select compounds with reduced psychoactivity. This is known to be connected to the CB1 receptor; thus, targeting the CB2 receptor is a popular objective. CB1 receptors could be maintained as a target to design new compounds, and mixed CB1–CB2 ligands could be effective if they are able to not cross the BBB. Furthermore, targeting the ECS with agents that activate cannabinoid receptors or inhibitors of endogenous degrading systems such as fatty acid amide hydrolase inhibitors may have relevant therapeutic impact on tumor growth. Additional studies into the downstream consequences of endocannabinoid treatment are required and may illuminate other potential therapeutic targets.

Keywords: cancer, cannabinoid receptors, endocannabinoid system, endocannabinoids

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1. Introduction

The discovery of the endocannabinoid system (ECS) has initiated a novel field of pharmacological studies to reveal its therapeutic potential in several pathological conditions including cancer. The ECS consists of cannabinoid receptors, their endogenous ligands (endocannabinoids) and proteins for their synthesis and inactivation [1]. Two definitive cannabinoid receptors have been identified [1], CB1 mainly located in the CNS [2-4] and CB2 expressed by immune cells [5], although found also in rat [6] and human brain, where it was demonstrated to occur under pathological conditions [7,8]. More recently, the orphan receptor GPR55 was shown to function as a novel cannabinoid receptor [9] that might play a physiological role in lipid or vascular biology [10]. Cannabinoid receptors modulate ion channels [5] and several pathways involved in the control of cell proliferation and survival [11-13]. Endocannabinoids are released 'on demand' in response to diverse physiological and pathological stimuli [14]. The best known are anandamide (AEA) and 2-arachidonoylglycerol (2-AG). However, a group of *N*-acylethanolamides (oleylethanolamide, palmitoylethanolamide, homo-γ-linoleylethanolamide and docosatetraenylethanolamide) is also known. Some of these compounds behave as 'entourage compounds' regulating endocannabinoid functions through unknown

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mechanisms [15]. AEA has been implicated in several pathological processes by acting as a CB1/CB2 agonist and activating the GPR55 receptor [9]. Complex enzymatic cascades regulate endocannabinoid production and inactivation [1]; all the enzymes involved in these pathways are potential targets for pharmacological intervention in a wide range of diseases including cancer. Recent findings show that endocannabinoids affect cancer cell viability and invasiveness modulating the activity of proteins and nuclear factors involved in cell proliferation, differentiation and apoptosis [16,17]. Numerous plant-derived (tetrahydrocannabinol (THC), cannabidiol (CBD)), synthetic (WIN-55, 212 – 2, HU-210) and endogenous cannabinoids control cancer cell growth and death [18-21]. More importantly, cannabinoid administration to nude mice curbs the growth of various types of tumor xenografts, including lung carcinoma [22] glioma [23], thyroid epitelioma [24], lymphoma [25], skin carcinoma [26], pancreatic carcinoma [27] and melanoma [28]. Moreover, cannabinoids appear selective antitumor agents with good safety profile as suggested by a pilot clinical trial on patients with glioblastoma multiforme [29]. The aim of this review is to update the current knowledge of the ECS in cancer and highlight very recent findings on its modulation in several tumors, considered novel potential targets for drug development in cancer therapy.

2. Body

2.1 Endocannabinoid levels in cancer

The ECS is highly conserved among species and shows modulating activity on proteins and nuclear factors involved in cell proliferation, differentiation and survival. Endocannabinoid levels are finely modulated under physiological and pathological conditions. A transient increment appears to be an adaptive reaction to restore cell homeostasis when this is acutely perturbed. For instance, high AEA concentrations were found in placenta, umbilical vein and plasma from maternal circulation for pregnancy maintenance and parturition [30]. However, in some chronic conditions, the alteration of the ECS seems to contribute to the progress and symptoms of neurodegenerative diseases [31]. Elevated levels of endocannabinoids have been reported in several tumors compared to the respective healthy tissue, for example, in glioblastoma, meningioma [32], pituitary adenoma [33], prostate [34], colon carcinoma and endometrial sarcoma [35-37] (**Table 1**). Both AEA and 2-AG are increased in human colorectal adenomatous polyps and carcinomas compared to normal colorectal mucosa [35]. AEA levels were enhanced in glioblastomas while 2-AG levels were increased in meningiomas when compared with human non-tumor brain tissue [32]. However, the levels of endocannabinoids were found to differ depending on the grade of tumor [36]. Compared with normal tissues, AEA levels remained unchanged in pancreatic ductal carcinoma pancreas [38], ileum lymphoma and bladder carcinoma while it decreased in stomach carcinoma [36]. Mass spectrometry analysis of endometrial carcinoma extracts showed significant increase of 2-AG levels in comparison with samples from healthy patients [39].

2.2 Endocannabinoid degrading system and cancer

A local endocannabinoid tone is an important factor to control the malignancy of different cancer cells. Thus, modulation of the levels of endocannabinoids, by use of inhibitors of endocannabinoid synthesis or metabolism, results in a change in the invasive properties of cancer cells in a manner consistent with a protective effect of endocannabinoids.

After synthesis, AEA is rapidly hydrolyzed and degraded by the enzyme fatty acid amide hydrolase (FAAH) [40] while monoacylglyceride lipase (MAGL) is the hydrolytic enzyme which degrades the 2-AG (**Figure 1**). MAGL has been recently found highly expressed in aggressive human cancer cells and primary tumors, including melanoma, ovarian and breast cancer, where it regulates a lipid network enriched in protumorigenic signaling molecules [41]. A correlation between FAAH and cancer has been primarily investigated in prostate adenocarcinomas, where tumor epithelial FAAH–immunoreactivity has been recently associated with prostate cancer severity and outcome [42,43] and in pancreatic ductal adenocarcinomas where a correlation of high FAAH/MAGL levels and survival has been observed [38]. Therefore, blocking the degradation pathway and in particular the FAAH activity by specific inhibitors may enhance the anti-proliferative effects of AEA and have beneficial effects in cancer treatment. Indeed, treatment of human breast cancer cells *in vitro* with palmitoylethanolamide reduced up to 30 – 40% FAAH expression thereby allowing the accumulation of AEA and increasing its anti-proliferative effects [44].

In addition, treatment of athymic mice with thyroid tumor xenografts with VDM-11 (a selective inhibitor of endocannabinoid cellular reuptake) or arachidonyl–5-HT (a selective blocker of endocannabinoid hydrolysis) increased the intra-tumor levels of AEA and significantly decreased tumor volume [19]. Of note, FAAH pharmacological inhibition or siRNA knockdown decreases prostate cancer cell invasion [43]. Inhibitors of these enzymes have demonstrated therapeutic benefit in animal models of other several disorders too, including neuropathic pain, anxiety and inflammatory bowel diseases [45]. Of note, in a lipopolysaccharide (LPS) mouse model of inflammatory pain, FAAH(-/-) mice exhibit reduced edema and hyperalgesia. Through the same mechanism, the FAAH inhibitor URB597 attenuates the development of LPS-induced edema and hyperalgesia and more importantly reduces the production levels of IL-1β and TNF-α pro-inflammatory cytokines, offering a potentially powerful strategy to treat chronic inflammatory pain syndromes [46].

2.3 Cannabinoid receptors and cancer

Several studies suggested that the cannabinoid receptor system is altered during carcinogenesis. Cannabinoid

Table 1. Levels of endocannabinoids and CB receptors in malignant vs normal cells.

Levels of CB receptors	Tumor type	Levels of endocannabinoids	Ref.
↑ **CB1 and CB2 receptors**	Mantle cell lymphoma	↑ AEA	[47]
	Acute myeloid leukemia	↑ AEA	[48]
	Well-differentiated hepatocellular carcinoma	↑ AEA	[49]
	Prostate carcinoma	↑ 2-AG; ↓ AEA	[50]
	Malignant astrocytoma	↑ AEA	[51]
	Pancreatic carcinoma	= AEA and 2-AG	[27]
↓ **CB1 and CB2 receptors**	Poorly differentiated hepatocellular carcinoma	↑ AEA	[52]
CB1 and CB2 receptors similar to control levels	Kaposi's sarcoma		[53]
	Non-melanoma skin cancer		
	Astroglial carcinoma	↑ AEA	[34]
	Pituitary adenoma	↑ AEA and 2-AG	[54] [33]
↑ **CB2 receptors**	Glioblastoma	↑ AEA	[55]
	Meningioma	↑ 2-AG	[32]
	Estrogen receptor-negative primary breast carcinoma	↑ AEA	[56]
	Endometrial carcinoma	↑ 2-AG	[39]
	Colon carcinoma	↑ AEA and 2-AG	[35,59]
↑ **CB1 receptors**	Rhabdomyosarcoma		[58]
	Gastrocarcinoma	↓ AEA	[57]
↓ **CB1 receptors**	Primary breast carcinoma	↑ AEA	[25]
↑ **TRPV1 receptors**	Prostate carcinoma	↑ 2-AG; ↓ AEA	[50]
	Squamous cell carcinoma of the human tongue		[61]

2-AG: 2-Arachidonoylglycerol; AEA: Anandamide; CB: Cannabinoid.

receptors play a key role in endocannabinoid antitumor effects; however, the regulation of their expression in cancer tissues is a topic not sufficiently investigated. It seems that the levels of cannabinoid receptors are differently regulated in normal versus malignant cells (**Table 1**). This pattern of expression seems to be a common mechanism of protection of normal cells from pro-apoptotic and anti-proliferative effects of cannabinoid agonists [17]. Increased expression of CB1 and CB2 has been reported in mantle cell lymphoma [47], acute myeloid leukemia [48], hepatocellular carcinoma [49], prostate cancer cell lines [50], malignant astrocytomas [51] and human pancreatic cancer [27] compared to normal tissue (**Table 1**). 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 [52]. Instead, the levels of both receptors were similar to healthy tissue in Kaposi's sarcoma cells [53], non-melanoma skin cancer [34], pituitary adenomas [33] and astroglial tumors [54]. In several types of tumor, a remarkable increase of CB2 receptors levels has been detected, such as in endothelial cells of glioblastoma [55], in primary breast cancer compared with normal breast tissue [25], in estrogen receptor (ER)-negative breast tumors compared with ER-positive tumors [25] and in 91% of ErbB2-positive breast tumors (**Table 1**) [56]. Other types of tumors are instead accompanied by overexpression of the CB1 receptor, such as in the parietal cells of human gastric mucosa and in human gastric cancer cell HGC-27 [57]. Indeed, gene expression profiling revealed that the gene coding for CB1 is highly upregulated in rhabdomyosarcoma biopsies (**Table 1**) [58]. Significant levels of CB1 and CB2 receptors were found in both normal and colorectal cancer tissue. It was observed that AEA and 2AG antiproliferative effect in colon cancer cells was mediated by CB1 activation [35]; however, an anti-proliferative role associated with CB2 has also been proposed in the same study. A recent study [59] also confirmed that human colorectal cancer specimens and the corresponding normal colonic mucosa express CB1 and CB2 receptors at the mRNA and protein levels as previously reported (**Table 1**) [35]. Although the antitumor effects of (endo)cannabinoid can be mediated by cannabinoid receptors, the role of these receptors in cancer is still unclear; however, their levels of expression could have a prognostic value alone or in association with other recognized prognostic markers. CB2 receptor expression positively correlates with the histological grade of breast cancer [60]. In addition, high pancreatic cancer cell level of CB1 is associated with shorter survival, together with low FAAH and MGLL levels [38]. A role also for the transient vanilloid receptor TRPV1 has been suggested in cancer; increased expression of this receptor was found in human

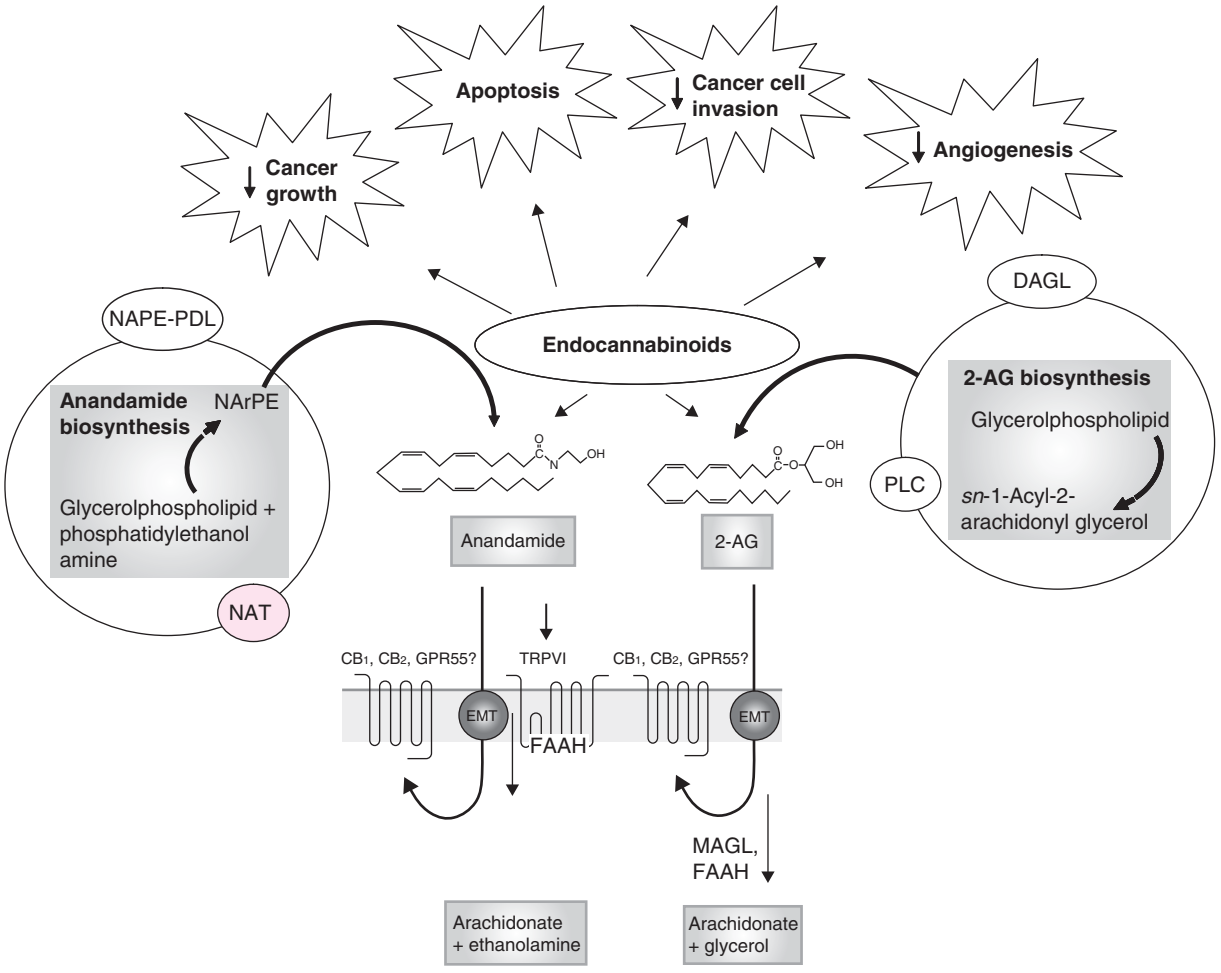

Figure 1. Anabolic and catabolic pathways of endocannabinoids and effects on cancer growth. The anandamide biosynthesis occurs through enzymatic hydrolysis of the membrane lipid precursor, NArPE. The anandamide biosynthetic enzymes NAT and NAPE-PLD and the inactivating enzyme FAAH are all located on intracellular membranes. The enzymes for 2-AG biosynthesis are the PLC and the sn-1-selective DAGLs mostly localized on the plasma membrane. After biosynthesis, endocannabinoids can activate cannabinoids receptors, either after previous release into the extracellular space or directly moving within the cell membrane. EMT seems to facilitate both endocannabinoid release and re-uptake. The enzyme FAAH hydrolyzes anandamide and related compounds in arachidonate and ethanolamine and MAGL hydrolyzes 2-AG in arachidonate and glycerol. Endocannabinoids might directly inhibit tumor growth by mechanisms involving induction of apoptosis, inhibition of cancer cell invasion and inhibition of neo-angiogenesis.

2-AG: 2-Arachidonoylglycerol; DAGL: Diacylglycerol lipase; EMT: Endocannabinoid membrane transporter; FAAH: Fatty acid amide hydrolase; MAGL: Monoacylglyceride lipase; NAPE-PLD: *N*-acylphosphatidyl-ethanolamine-specific phospholipase D; NArPE: *N*-arachidonoyl phosphatidylethanolamine; NAT: *N*-acyltransferase; PLC: Phospholipase C.

prostate carcinoma [50] and in squamous cell carcinoma (SCC) of the human tongue compared to normal tongue. However, statistical analysis revealed that the marked overexpression of TRPV1 found in all grades of SCC showed no correlation with the degree of malignancy of the tumors [61].

At present, little is known about the regulation of cannabinoid receptor expression in tumor tissue; the different response of normal and malignant cells to cannabinoids and the abnormal expression of cannabinoid receptors in cancer call for further research on the regulation of cannabinoid receptors.

3. Antitumor mechanisms of endocannabinoids and their derivates

The activation of cannabinoid receptors on tumor cells modulates signaling pathways involved in cell proliferation and survival, and although the downstream events are not completely unraveled, there is substantial evidence for the involvement of at least four mechanisms: antiproliferative action through the suppression of mitogenic signal, induction of apoptosis, inhibition of tumor cell invasion and neo-angiogenesis.

3.1 Anti-proliferative effects

Numerous pharmacological studies have demonstrated the anti-proliferative action of endocannabinoids and their derivates in several tumor models through cannabinoid receptor-dependent or -independent mechanisms that may involve cytotoxic or cytostatic effects, apoptosis induction and anti-metastatic effects. AEA exerts anti-proliferative effects on cholangiocarcinoma [62], human thyroid carcinoma [63], hepatocellular carcinoma [49], COX-2 overexpressing non-melanoma skin cancer [64] and breast cancer proliferation; this effect on mammary tumors is accompanied by reduced levels of the long form of the prolactin receptor [65] and trk nerve growth factor (NGF) receptor [66]. These effects are due to cell cycle arrest with activation of Chk1 and suppression of Cdk2 activity [67]. Furthermore, the AEA analog R(+)-methanandamide (MET-A) induces growth inhibition and apoptosis in prostate cancer PC-3 cells through CB2-mediated signaling [68] and decreases the EGFR expression [69]. The CB1–CB2 agonist, WIN-55,212 – 2, induces gastric cancer cell apoptosis [70], WIN-55,212 – 2 and the CB2 synthetic agonist JWH-133 inhibit human breast cancer cell proliferation [25]. Indeed, *in vivo* studies reported that administration of endocannabinoids induces the regression of a broad array of cancer types, such as thyroid epitheliomas through CB1 receptors [24], lymphomas [71], gliomas [15], skin carcinomas [26] and pancreatic cancer [27] through CB2 receptors. AEA suppresses rat C6 glioma cell growth; this effect was partially blocked by the CB1, CB2, and TRPV1 antagonists, but was completely attenuated when these antagonists were added in combination [72]. Of note, on human glioma cells, CBD exerts anti-proliferative effects correlated to induction of apoptosis, not reverted by cannabinoid and vanilloid receptor antagonists [73].

3.2 Pro-apoptotic effects

Apoptosis induced by cannabinoids can involve caspase-dependent and -independent pathways. For instance, the CB1 receptor antagonist, rimonabant (SR141716), inhibits leukemia cell growth promoting apoptosome-complex formation and caspase pathway activation. [74]. Moreover, several studies confirmed pro-apoptotic and antitumor effects of cannabinoids via mechanisms involving *de novo* synthesis of ceramide [23]. For instance, in colorectal cancer cells, CB1 activation mediated induction of apoptosis, through inhibition of RAS-MAPK and PI3K-AKT pathways, downregulation of anti-apoptotic factors and activation of ceramide [75]. CB1 and CB2 receptor activation stimulates ceramide *de novo* synthesis in different human tumors such as glioma, pancreatic cells, leukemia and mantle cell lymphoma. AEA can induce growth suppressive/pro-apoptotic effects in cholangiocarcinoma cells via stabilization of the lipid raft structures within the plasma membrane, increased production of ceramide and subsequent recruitment of death receptor complex components into the lipid raft structures through activation of the non-canonical Wnt signaling pathway [62].

3.3 Effects on tumor invasion

Modulation of cancer cell invasion has recently emerged as a topic of increasing interest. MMPs are emerging as a family of enzymes that exert important functions during tumor invasion. Tissue inhibitors of MMPs (TIMPs), and in particular TIMP-1, have been shown to inhibit the proteolytic activity of MMPs and suppress vascular tumor growth and angiogenesis in xenograft animal models [76]. For instance, the effects of AEA on MMP and TIMP expression were evaluated in various cancer cells. Using a cervical cancer cell line, Met-AEA as well as Δ9-THC and CBD inhibited the invasive properties of these cells via increased expression of TIMP-1 [77,78]. Inhibitory properties of endocannabinoids in tumor migration have also been described. AEA inhibited migration of colon carcinoma cells [48]; in an *in vivo* model of metastatic spreading using breast cancer cells, Met-AEA significantly reduced the number and dimension of metastatic nodes, an effect inhibited by specific CB1 receptor antagonists [18]. Indeed, there was a marked decrease in the phosphorylation of focal adhesion kinase and src, both of which are involved in the metastatic formation and development.

3.4 Effects on angiogenesis

Several studies suggest that angiogenesis is also regulated by cannabinoids. The AEA analog 2-methylarachidonyl-2′-fluoro-ethylamide (Met-F-AEA) has been shown to reduce sprout number and length of endothelial cell spheroids, inhibit capillary-like tube formation and suppress angiogenesis in the *in vivo* chick chorioallantoic membrane model [79]. Other studies indicate anti-angiogenic effects of cannabinoids related to the expression of VEGF. Met-F-AEA was found to decrease VEGF and VEGFR-1 levels in K-ras-transformed thyroid cells and experimental tumors of nude xenograft mice [80].

The effects described on endocannabinoid involvement in different stages of tumor progression support the emerging notion that targeting the ECS through drugs aimed at regulating this system may represent useful therapeutic tools for cancer treatment.

4. Signaling pathways involved in endocannabinoid anticancer effects

Anticancer effects elicited by (endo)cannabinoids can be mediated by CB1, CB2 or transient vanilloid TRPV1 receptors, but in some cases other mechanisms can be involved (e.g., COX, lipid rafts, PPAR-γ) [81]. AEA has been shown to inhibit breast cancer cell proliferation by acting via the CB1 receptor to activate the cAMP/PKA/MAPK pathway [80]. These effects ultimately result in decreased expression of prolactin receptor [65] and trk NGF receptor [66], with blocking of cell cycle progression by activation of checkpoint kinase Chk1 or cyclin-dependent kinase inhibitor p27/kip1 [80] and suppression of Cdk2 activity [67]. Furthermore, AEA through a CB1-dependent mechanism reduces the expression of

EGFR in several human prostatic cancer cell lines, a phenomenon that has been associated with G1 arrest and cell death [69] possibly by an increase of ceramide production. Similarly, the stimulation of CB1 receptor by Met-F-AEA inhibits the growth of xenograft tumors of thyroid origin by affecting the expression of VEGF tyrosine kinase receptor by blocking the activity of p21ras [80]. AEA may activate either CB2 or TRPV1 to elicit a similar response, although the mechanism by which this occurs is not clear. Recently, HMG-CoA reductase has been suggested as a new target of antitumor effect of AEA. In particular, the decreased activity of this enzyme is responsible for the inhibition of farnesylation of Ras oncogenic protein involved in cell proliferation of human mammary carcinoma cell lines [82]. Cannabinoids can also induce apoptosis via pathways not mediated by cannabinoid- or vanilloid receptor activation. In particular, in human H4 neuroglioma cells the endocannabinoid analog R(+)-methanandamide (R(+)-MA) has been shown to induce apoptosis through a pathway linked to lipid raft microdomains involving increased synthesis of pro-apoptotic sphingolipid ceramide. These effects involve COX-2 expression and subsequent formation of prostaglandin E2 via activation of p38 and p42/44 MAPKs [83,84]. Interestingly, increased Cox-2 expression is fundamental to sensitize the apoptosis-resistant colon cancer cells to AEA-induced cell death [85]. In the same way, AEA induces cell death in Cox-2 overexpressing squamous carcinoma cells but not in keratinocytes, which express low basal level of Cox-2. Resistance to AEA-mediated cell death in these cells was then reversed by overexpression of Cox-2 [86]. Alternatively, AEA-induced ceramide production can facilitate the Fas/FasL death receptor complex within the lipid raft structure, resulting in increased apoptosis of cholangiocarcinoma cells [87]. A change in cytosolic free $Ca2^+$ levels is a pivotal signal for various cellular responses. Recent studies suggested that AEA induced apoptosis in human osteosarcoma cells by causing $Ca2^+$ influx and release that converge on p38MAPK phosphorylation and subsequent activation of caspase-3 leading to apoptosis [88].

The pro-apoptotic effects of (endo)cannabinoid converge on ceramide accumulation and activation of (ER) stress-related pathway. The stress-regulated protein p8 plays a key role in this effect controlling the expression of ATF-4, CHOP and TRB3 factors [27]. This cascade of events triggers the activation of mitochondrial intrinsic apoptotic pathway through mechanisms not unraveled yet. However, it has been suggested that the pro-apoptotic activity of (endo)cannabinoids may involve the JNK activation [68] as well as the inhibition of both RAS–MAPK/ERK and PI3K–AKT survival signaling cascades, accompanied by the activation of the pro-apoptotic BCL-2 family member Bad. Very recently, it was demonstrated that non-psychoactive CB2-selective agonists such as JWH-133 were efficient to treat highly aggressive and low responsive tumors of MMTV-neu mice, a clinically relevant animal model of ErbB2-positive breast cancer [56]. This antitumor action relies, at least partially, on the inhibition of the pro-tumorigenic PI3K–Akt pathway, whose importance in breast cancer is corroborated by clinical studies showing in most ErbB2-overexpressing tumor activation of Akt. A recent publication furthermore demonstrated the induction of autophagy by cannabinoids. In human glioma cells, THC induces ceramide accumulation and eukaryotic translation initiation factor 2α phosphorylation; thereby, activated ER stress response promoted autophagy via TRB3-dependent inhibition of the Akt–mammalian target of rapamycin complex 1 axis [89]. Interestingly, the effects of cannabinoids on the Notch transmembrane receptor family that play a pivotal role in cellular differentiation, proliferation and apoptosis have been recently demonstrated. In particular, AEA and 2-AG have opposite effects on cholangiocarcinoma growth, depending on the differential activation of Notch signaling that requires the γ-secretase complex for activation. Anti-proliferative effects of AEA are associated with increased Notch 1 mRNA, presenilin 1-dependent proteolytic cleavage and activation, and nuclear translocation while the growth promoting effects of 2-AG are associated with presenilin 2-dependent activation of Notch 2 signaling [90]. Furthermore, AEA inhibits angiogenesis, tumor cell migration and invasion. Met-AEA inhibits angiogenesis via decreased VEGF expression and inhibition of the basic fibroblast growth factor-stimulated endothelial cell proliferation [79]. Of note, in thyroid tumors the same compound inhibits the angiogenetic process via downregulation of the pro-angiogenic growth factor VEGF and its receptor Flt-1 expression, thereby counteracting the cancer growth *in vivo* [80]. Regarding the AEA-induced inhibition of migration, it is probably due to decreased activation in breast cancer cells of focal adhesion kinases and src kinase [18]. Met-AEA also inhibits human cervical cancer (HeLa) cell invasion by decreasing the expression of proteins responsible for breaking down the extracellular matrix of the target organ, such as MMPs and increasing the expression of TIMPs. These effects were reversed by specific inhibitors of p38 and p42/44 MAPKs [77] suggesting involvement of this signaling pathway. Finally, in androgen-independent prostate cancer cells, endogenous 2-AG and CB1 agonists reduced invasion through CB1-dependent inhibition of adenylyl cyclase, decreasing phospho-kinase A activity [34].

5. Preclinical/clinical use of endocannabinoid-derived drugs: beneficial and undesired effects

The ECS modulation is involved in various pathophysiological conditions in both central and peripheral tissues and is implicated in the hormonal regulation of food intake and energy metabolism, cardiovascular, gastrointestinal, immune, behavioral, anti-proliferative and mammalian reproduction functions. Intense research efforts yielded numerous drugs interacting with the main elements of the ECS, cannabinoid receptor agonists and antagonists, inhibitors of FAAH and

MAGL and diacylglycerol lipases. Therapeutically relevant ECS modulators include THC in combination with CBD (sativex), its synthetic forms (marinol), and its closely related compounds (cesamet). These compounds remain the only clinically applied cannabinoid receptor agonists. The principal pharmacological effects of these compounds include analgesia, muscle relaxation, anti-emesis, appetite stimulation and psychoactivity [91]. Rimonabant is the first CB1 blocker launched to treat cardiometabolic risk factors in obese and overweight patients. Phase III clinical trials showed the drug's ability to regulate intra-abdominal fat tissue levels, lipidemic, glycemic and inflammatory parameters [92]. However, safety concerns led to its withdrawal, as probably interfering with the 'autoprotective' role of the ECS it induced ansiogenic and pro-depressant effects. Considering that many side effects rely on CNS action, it would be adequate for the development of peripherally-restricted antagonists to be unable to cross the BBB or to use partial CB1 agonists in order to limit adverse effects. A different approach could be the use of phytocannabinoids such as CBD [93] with very weak or no psychotropic effects. Cannabinoid receptor ligands are potential therapeutic agents in neurodegenerative diseases such as Parkinson and Alzheimer and AIDS-related neurodegeneration [94]. Cannabinoids represent also a novel class of anti-inflammatory drugs due to their ability to negatively impact the release of inflammatory mediators and the induction of pro-inflammatory transcriptional programs. Of note, the activation of CB2 receptor is a key factor of the cannabinoid suppressive effects, while apparently pro-inflammatory effects seem to be attributable to the CB1 receptor expression [95]. Given the growing evidence of the expression of the CB1 receptor on numerous immunocytes, it is likely that the clinical promise of cannabinoids as anti-inflammatory drugs may rely on the development of highly selective CB2 agonists. In terms of clinical application, the inhaled or ingested THC can acutely suppress ongoing airway or gastrointestinal inflammation, leading to particular interest in the development of cannabinoid therapeutics for treatment of asthma, chronic obstructive pulmonary disease and Crohn's disease/IBD.

An emerging research area is represented by the role of ECS elements in mammalian reproduction given their implication in oogenesis, embryo oviductal transport, blastocyst implantation, placental development and pregnancy outcomes, and sperm survival, motility, capacitation and acrosome reaction [96].

5.1 Preclinical evidence of cannabinoid efficacy in cancer

Cannabinoid-related drugs are emerging as valuable adjunctive agents for the management of multiple symptoms of cancer and therapy-induced side effects. Available data support a broad spectrum of palliative properties, including appetite stimulation, inhibition of nausea and emesis associated with chemotherapy or radiotherapy, pain relief, mood amelioration and relief from insomnia [81]. Marinol, cesamet and sativex have been already approved by the FDA for these indications. Recently, results of multi-center, double-blind, randomized, placebo-controlled, parallel-group study showed the efficacy, safety and tolerability of THC:CBD extract and THC extract in 177 patients with intractable cancer-related pain. This study shows that THC:CBD extract is a more promising efficacious treatment than THC extract alone for relief of advanced cancer pain in patients not achieving an adequate analgesic response to opioids [97]. However, side effects of cannabinoids are known: euphoric mood alteration, sedation and alleviating inhibition but also disphoric mood alteration including anxiety, panic and psychosis. Until now, the use of cannabinoid-derived drugs has shown a fair safety profile with respect to current chemotherapeutics. However, only a single pilot Phase I–II clinical trial, approved by the Spanish Ministry of Health in 2002, has been performed so far and the results have been recently disclosed [98]. This study evaluated the safety profile of THC intracranial administration and its antitumor efficacy in a cohort of nine terminal patients affected by recurrent glioblastoma multiforme, an aggressive primary brain tumor with poor prognosis and no efficacious treatment. Cannabinoid delivery resulted as safe without psychoactive effects and median survival was prolonged by 24 weeks. Indeed, THC decreased tumor cell proliferation and increased apoptosis when administered to two patients. Although this pioneer study suffers from several limitations, good safety profile of THC was demonstrated. To optimize the results, the protocol should also involve a larger cohort of patients and combinatorial studies with commonly used chemotherapeutics. Finally, a non-invasive, less traumatic administration route would be more desirable for clinical practice.

6. Conclusion

Findings widely reported in literature support the regulatory action elicited in health and pathological conditions by the ECS. The modulation of both endocannabinoid levels and their receptors in cancer has prompted the development of numerous (endo)cannabinoid derived agents aimed to target cannabinoid receptors or modulate the enzymes involved in endocannabinoid production or degradation. Thus, myriad effects on tumor models both *in vitro* and *in vivo* have been reported suggesting antitumor properties such as inhibition of cancer cell growth, induction of apoptosis and block of processes involved in tumor progression, such as angiogenesis, and cell migration. These effects might involve several signaling pathways being both cannabinoid receptor-dependent or -independent. Overall research has led to the use of some of these compounds in clinical trials, and although only few data are available, these studies strongly suggest the safety profile of cannabinoid derivatives. The proposed antitumor efficacy of (endo)cannabinoid-related drugs alone or in combination with other currently used chemotherapeutics is not completely investigated and needs a deeper research at both

preclinical and clinical level in order to allow a safe translation into the clinical setting.

7. Expert opinion

Key findings from current literature show that the study of the ECS is emerging as a relevant topic to be targeted in cancer. However, research performed up to date is still at basic or preclinical levels but we believe in the high potential of multiple studies.

One of the issues that should be pursued is to better select compounds in order to reduce psychoactivity known to be connected to the CB1 receptor; thus, for example, focusing on CB2 receptor as a primary target is becoming the objective of ongoing studies that could probably lead to new highly selective compounds. The design of novel CB2 ligands with high affinity at the CB2 receptor could reduce side effects due to high doses; thus, design studies could identify promising candidates to test *in vitro* and *in vivo* models and in clinical trials in the near future. Furthermore, CB1 receptors could be maintained as a target to design new compounds and or mixed CB1–CB2 ligands only if these substances are able to not cross the BBB. In addition, phytocannabinoids could be considered as potential candidates to pursue studies on clinical trials; these substances are able to maintain cannabinoid anticancer effects and indeed present very low psychoactivity, and their efficacy is supported by the applications of CBD in several clinically applied drugs. Research in this field requires further studies to better characterize the molecular mechanisms on which anticancer effects of (endo)cannabinoids are based, exhaustive clinical trials to establish the real advantage with respect to currently used chemotherapeutic or combinatory studies in order to reduce undesired side effects. Another relevant issue that probably will be the objective of studies is the regulation of cannabinoid receptor in carcinogenesis and the possibility of being considered as a potential marker of tumor progression. The aim of these studies should be the evaluation of cannabinoid receptor expression in tumor versus normal tissues in order to achieve significant antitumor efficacy without immunosuppression. Furthermore, targeting the ECS with agents that activate cannabinoid receptors or inhibitors of endogenous degrading systems such as FAAH inhibitors may be of relevant therapeutic impact on tumor growth and spreading; indeed, the increase of the endogenous levels of endocannabinoids may prove to be beneficial in the treatment of various cancers. Further studies into the downstream consequences of endocannabinoid treatment are required and may illuminate other potential therapeutic targets. The potential related to the study of the ECS is high considering also that preclinical study and clinical trials revealed the safety profile of cannabinoid derived drugs; thus, improving the studies on this field could show not only the efficacy of such compounds as palliative and antinociceptive drugs but also potential application as therapeutic agents, probably taking to new advances in cancer research.

Declaration of interest

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•• **Of considerable importance, this work is the only pilot Phase I – II clinical trial performed so far on cannabinoids.**

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