CHAPTER TWELVE

Cannabinoids as Anticancer Drugs

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Abstract

The endocannabinoid system encompassing cannabinoid receptors, endogenous receptor ligands (endocannabinoids), as well as enzymes conferring the synthesis and degradation of endocannabinoids has emerged as a considerable target for pharmacotherapeutical approaches of numerous diseases. Besides palliative effects of cannabinoids used in cancer treatment, phytocannabinoids, synthetic agonists, as well as substances that increase endogenous endocannabinoid levels have gained interest as potential agents for systemic cancer treatment. Accordingly, cannabinoid compounds have been reported to inhibit tumor growth and spreading in numerous rodent models. The underlying mechanisms include induction of apoptosis, autophagy, and cell cycle arrest in tumor cells as well as inhibition of tumor cell invasion and angiogenic features of endothelial cells. In addition, cannabinoids have been shown to suppress epithelial-to-mesenchymal transition, to enhance tumor immune surveillance, and to support chemotherapeutics' effects on drug-resistant cancer cells. However, unwanted side effects include psychoactivity and possibly pathogenic effects on liver health. Other cannabinoids such as the nonpsychoactive cannabidiol exert a comparatively good safety profile while exhibiting considerable anticancer properties. So far experience with

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anticarcinogenic effects of cannabinoids is confined to in vitro studies and animal models. Although a bench-to-bedside conversion remains to be established, the current knowledge suggests cannabinoid compounds to serve as a group of drugs that may offer significant advantages for patients suffering from cancer diseases. The present review summarizes the role of the endocannabinoid system and cannabinoid compounds in tumor progression.

ABBREVIATIONS

2-AG 2-arachidonoylglycerol
AA-5HT *N*-arachidonoyl serotonin
ABCG ATP-binding cassette, subfamily G
ACPA arachidonoyl cyclopropylamide
AEA *N*-arachidonoylethanolamine, arachidonoylethanolamide, anandamide
CB_1 cannabinoid receptor 1
CB_2 cannabinoid receptor 2
CBD cannabidiol
CBN cannabinol
CD cluster of differentiation
Cdc25A cell division cycle 25 homolog A
Cdk2 cyclin-dependent kinase 2
JNK c-Jun NH_2-terminal kinase
DAG lipase diacylglycerol lipase
DAG diacylglycerol
EGF epidermal growth factor
EMT epithelial-to-mesenchymal transition
FAAH fatty acid amide hydrolase
GPR G protein-coupled receptor
HER human epidermal growth factor receptor
HUVEC human umbilical vein endothelial cells
ICAM-1 intercellular adhesion molecule-1
Id inhibitor of DNA binding
LAK lymphokine-activated killer cells
LFA-1 lymphocyte function-associated antigen-1
MAGL monoacylglycerol lipase
MAPK mitogen-activated protein kinase
Met-F-AEA 2-methyl-2′-F-anandamide
MMP matrix metalloproteinase
mRNA messenger RNA
NAPE *N*-acyl-phosphatidylethanolamine
NGF nerve growth factor
OEA *N*-oleoylethanolamine, oleoylethanolamide
PAI-1 plasminogen activator inhibitor-1
PEA *N*-palmitoylethanolamine, palmitoylethanolamide
p-GP p-glycoprotein
PLD phospholipase D
PPAR peroxisome proliferator-activated receptor

ROS reactive oxygen species
SDF1α stromal cell-derived factor-1α
SEA *N*-stearoylethanolamine, stearoylethanolamide
siRNA small interfering RNA
THC Δ^9-tetrahydrocannabinol
TIMP-1 tissue inhibitor of matrix metalloproteinases-1
TRPM8 transient receptor potential melastatin type-8
TRPV transient receptor potential vanilloid
VEGF vascular endothelial growth factor

1. INTRODUCTION

The first study aiming at cannabinoid compounds as possible anticancer agents was published in the 1970s far before the discovery of cannabinoid receptors. With their initial experiments, Munson et al. were able to demonstrate that Δ^8-tetrahydrocannabinol, Δ^9-tetrahydrocannabinol (THC), and cannabinol (CBN) suppress tumor growth and prolong the survival of mice in a Lewis lung adenocarcinoma model (Munson, Harris, Friedman, Dewey, & Carchman, 1975). Three years later, the same research group presented data that suggested cannabinoids to exhibit stereospecific binding to biological materials (Harris, Carchman, & Martin, 1978). Notably, at that time cannabinoids were rather supposed to interact with steroid-binding sites than having their own specific cannabinoid target structures. Accordingly, THC was found to interfere with estrogen receptor activation (Rawitch, Schultz, Ebner, & Vardaris, 1977). From the current point of view, the interference of cannabinoids with estrogen action may at least partly be explained by an estrogen response element in exon 1 of the gene encoding the cannabinoid receptor 1 (CB_1) and a cannabinoid-induced upregulation of estrogen receptor-β (Proto et al., 2012; Zhang et al., 2004). However, some investigators also found several cannabinoid compounds to lack interference with estrogen receptors (Ruh, Taylor, Howlett, & Welshons, 1997).

In the 1990s, the Gi/o-coupled cannabinoid receptors, referred to as CB_1 and CB_2 (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990; Munro, Thomas, & Abu-Shaar, 1993), as well as endogenously synthesized fatty acid derivatives acting at these receptors, i.e., arachidonoylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), have been identified (Devane et al., 1992; Mechoulam et al., 1995). Further

investigations unraveled the formation of AEA by enzymatic turnover of *N*-arachidonoyl-phosphatidylethanolamine by phospholipase D (PLD) (Di Marzo et al., 1994) and of 2-AG by cleavage of membrane phospholipids via phospholipase C as well as by turnover of diacylglycerol (DAG) via diacylglycerol lipase (DAG lipase) (Stella, Schweitzer, & Piomelli, 1997). In this context, the serine hydrolase fatty acid amide hydrolase (FAAH) has been demonstrated as catabolic enzyme for the degradation of AEA (Deutsch & Chin, 1993) as well as of 2-AG (Di Marzo, Bisogno, Sugiura, Melck, & De Petrocellis, 1998; Goparaju, Ueda, Yamaguchi, & Yamamoto, 1998). Although lacking affinity to cannabinoid receptors, the endocannabinoid-like substances oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) are likewise hydrolyzed by FAAH (Saghatelian et al., 2004). Later, the monoacylglycerol lipase (MAGL) was discovered as the major enzyme of 2-AG degradation (Blankman, Simon, & Cravatt, 2007).

In addition to the specific cannabinoid receptors CB_1 and CB_2, the cation channel transient receptor potential vanilloid 1 (TRPV1) has been reported as additional receptor activated by AEA (Zygmunt et al., 1999) and by the phytocannabinoid cannabidiol (CBD) (Bisogno et al., 2001). Another receptor of the TRP family triggered by the phytocannabinoid cannabigerol is the transient receptor potential melastatin type-8 (TRPM8) (Borrelli et al., 2014). Additionally, CBD has been demonstrated to elicit apoptosis in prostate cancer cells partly via antagonism at the TRPM8 (De Petrocellis et al., 2013).

Meanwhile, several studies have suggested additional G protein-coupled receptors (GPR) to be involved in cannabinoid action such as GPR55 which is antagonized by CBD and activated by abnormal-CBD (synthetic regioisomer of CBD), the specific GPR55 agonist O-1602, R(+)-methanandamide (hydrolysis-stable analog of AEA), JWH-015 (CB_2 receptor agonist), as well as THC (Johns et al., 2007; Lauckner et al., 2008; Ryberg et al., 2007). As a further orphan receptor implicated in cannabinoid action, GPR119 was shown to be triggered by the endocannabinoid-like substances OEA and PEA (Overton et al., 2006). In addition, these endocannabinoid-like substances have been described as ligands at the intracellular receptor peroxisome proliferator-activated receptor-α (PPARα) (Artmann et al., 2008; Fu et al., 2003; Tellez et al., 2013). In this context, OEA was shown to induce satiety and to reduce body weight gain via PPARα activation (Fu et al., 2003).

Recently, TRPV2 was described as additional cannabinoid target. Accordingly, CBD was shown to enhance the sensitivity of glioblastoma and blood cancer cells toward chemotherapeutics via TRPV2 activation (Morelli et al., 2014; Nabissi, Morelli, Santoni, & Santoni, 2013). Moreover, TRPV2 has been demonstrated to mediate CBD-induced autophagy of glioblastoma cells (Nabissi et al., 2015).

The first comprehensive study that addressed cannabinoid receptors as platform for a growth-inhibitory action on cancer cells was published in 1998 (De Petrocellis et al., 1998). In subsequent years anticancer effects of cannabinoids were confirmed by in vitro and in vivo studies using glioblastoma cells (Galve-Roperh et al., 2000; Gomez del Pulgar, Velasco, Sanchez, Haro, & Guzman, 2002; Guzmán, Sanchez, & Galve-Roperh, 2001), followed by an avalanche of publications on anticarcinogenic effects of diverse cannabinoid compounds on various cancer entities.

Noteworthy, R(+)-methanandamide has also been reported to induce apoptosis in cancer cells via enhanced intracellular ceramide levels without involvement of cannabinoid receptors (Hinz, Ramer, Eichele, Weinzierl, & Brune, 2004a). In context with such receptor-independent effects lipid raft microdomains were demonstrated as initial platforms for the toxic impact of R(+)-methanandamide on neuroglioma cells (Hinz, Ramer, Eichele, Weinzierl, & Brune, 2004b). Other studies reported cannabinoids such as AEA to confer toxic effects toward head and neck squamous cell carcinoma cells via production of reactive oxygen species (ROS) regardless of cannabinoid receptor activation (Park et al., 2015). In this context, particularly CBD has been described as anticancer drug due to its capacity to act as a redox modulator thereby conferring inhibition of glioma cell proliferation (Singer et al., 2015) as well as of breast cancer growth and spreading (McAllister et al., 2011).

The main shortcoming and challenge with reference to bench-to-bedside conversion today, however, is the lack of clinical knowledge concerning the safety and efficacy of the cannabinoid dosing regimes that may yield systemic anticancer effects. Until today, merely a single clinical pilot study published in 2006 was carried out that indicated intracranially administered THC to be safe in glioblastoma patients (Guzmán et al., 2006). Other observations were only case reports that suggested, e.g., cannabinoid administration to be associated with a regression of septum pellucidum/forniceal pilocytic astrocytoma tumors in two children (Foroughi, Hendson, Sargent, & Steinbok, 2011) or to elicit beneficial effects on treatment of

terminal acute lymphoblastic leukemia with a Philadelphia chromosome mutation of a 14-year-old girl (Singh & Bali, 2013).

As a matter of fact, the clinical use of cannabinoid compounds may be limited by their property to activate central CB_1 receptors being associated with psychoactive effects. In addition, recent findings suggested activation of peripheral CB_1 receptors to confer a risk for the development of liver fibrosis (Teixeira-Clerc et al., 2006). In line with their psychoactive effects, a recent meta-analysis revealed nervous system disorders as most common adverse effects of cannabinoids. However, the authors of this study did not explicitly record liver diseases as serious adverse effect, probably due to the lack of larger studies required to characterize safety issues under conditions of long-term exposure (Wang, Collet, Shapiro, & Ware, 2008).

With respect to central adverse effects, the scientific focus was set on nonpsychoactive cannabinoids. Of particular interest in this context are CB_2 agonists that spare psychoactive effects and may further elicit beneficial effects on the liver by virtue of their antifibrotic action (Julien et al., 2005; Muñoz-Luque et al., 2008). JWH-133, a CB_2 receptor agonist, has been reported to exert tumor-regressive effects in glioblastoma (Sanchez et al., 2001) and skin cancer (Blazquez et al., 2003).

A further anticancerogenic cannabinoid with considerable efficacy in preclinical studies is the nonpsychoactive phytocannabinoid CBD that has been shown to confer a broad array of tumor-blocking properties such as inhibition of cancer cell proliferation (De Petrocellis et al., 2013; Ligresti et al., 2006), cancer cell invasion, and metastasis (Ramer, Merkord, Rohde, & Hinz, 2010; Ramer et al., 2012), as well as tumor angiogenesis (Ramer, Fischer, Haustein, Manda, & Hinz, 2014; Solinas et al., 2012). In addition, CBD has been described to cause apoptosis (Ramer et al., 2013), to enhance immune responses toward tumor cells (Haustein, Ramer, Linnebacher, Manda, & Hinz, 2014), and to sensitize cancer cells toward chemotherapeutics (Holland et al., 2006; Morelli et al., 2014; Nabissi et al., 2013). Finally, CBD was recently found to downregulate markers of epithelial-to-mesenchymal transition (EMT) (Soroceanu et al., 2013). Noteworthy in this context, inhibition of EMT was likewise presented for the cannabinoids WIN 55,212-2 (Xian et al., 2016) and AEA (Laezza, d'Alessandro, Malfitano, & Bifulco, 2013).

Taken together, the current knowledge suggests the endocannabinoid system as considerable target for pharmacological intervention conferring antineoplastic effects that cancer patients might benefit from. The following

sections summarize the role of the endocannabinoid system in tumor progression and highlight the options and risks of cannabinoid compounds as anticancer drugs.

2. MODULATION OF THE ENDOCANNABINOID SYSTEM IN TUMOR PROGRESSION

In past years, a number of studies were carried out to address the regulation of endocannabinoids, cannabinoid receptors, and endocannabinoid-degrading enzymes in cancer tissue. Data obtained from these investigations have raised several contradictory interpretations concerning the impact of the endocannabinoid system on cancer progression. The following section provides an overview on data obtained in this field.

2.1 Regulation of Endocannabinoids in Cancer Tissue

In agreement with the hypothesis of endocannabinoids as tumor growth-limiting endogenous substances, an early investigation found AEA and other acylethanolamides such as OEA, PEA, and stearoylethanolamide (SEA) at lower concentrations in meningiomas and gliomas as compared to healthy tissues (Maccarrone, Attinà, Cartoni, Bari, & Finazzi-Agrò, 2001). This finding was further substantiated by a study that reported decreased AEA levels in gliomas vs nontumor tissue (Wu et al., 2012). However, in the latter investigation, tissues of high-grade gliomas were found to contain more AEA than low-grade sections, and 2-AG appeared to be upregulated in glioma vs healthy tissue. Increased levels of AEA were observed in hepatocellular carcinoma vs tumor-free tissue (Mukhopadhyay et al., 2015). In addition, AEA and 2-AG concentrations were found to be upregulated in human meningiomas vs noncancerous brain tissue (Petersen et al., 2005) as well as in adenomatous polyps and colorectal carcinomas as compared to the healthy mucosa (Ligresti et al., 2003). Moreover, analyses of lipid extracts from endometrial carcinomas revealed an upregulation of 2-AG compared to biopsies from healthy patients, whereas AEA and PEA remained virtually unaltered (Guida et al., 2010). Finally, upregulation of endocannabinoids in cancer vs healthy tissue has been substantiated for prostate cancer (Nithipatikom et al., 2004; Schmid, Wold, Krebsbach, Berdyshev, & Schmid, 2002), pituitary adenomas (Pagotto et al., 2001), and colorectal cancer tissue (Chen et al., 2015).

On the other hand, the abovementioned pattern of AEA down-regulation and increased 2-AG levels in cancer tissue as published for gliomas (Wu et al., 2012) could be confirmed in analyses addressing circulating endocannabinoids. Accordingly, patients with different kinds of cancers had lower AEA and higher 2-AG plasma concentrations when compared to a control group (Sailler et al., 2014). The latter study further found increasing OEA levels to correlate with higher numbers of metastases. Another investigation substantiated this notion by detecting elevated serum 2-AG levels to be associated with diffuse large B-cell lymphoma. Here, serum 2-AG levels were significantly higher in cases with late stage disease (Zhang et al., 2016).

2.2 FAAH and MAGL Expression in Cancerous Lesions

The majority of studies that evaluated levels of FAAH and MAGL in cancer patients, revealed an even higher expression of these endocannabinoid-degrading enzymes in cancer vs healthy tissue.

Thus, higher expression levels of FAAH have been reported for biopsies obtained from patients with prostate cancer (Endsley et al., 2008). Another investigation found a correlation between FAAH expression and disease severity using a midrange, but not a high CB_1 level cutoff value (Thors et al., 2010).

Higher expression levels have been also reported for MAGL in ductal breast tumors when compared to less malignant medullary breast tumors (Gjerstorff et al., 2006). Furthermore, an increase of MAGL expression could be confirmed for high-grade primary ovarian tumors (Nomura et al., 2010) as well as for colorectal cancer compared to normal tissue (Ye et al., 2011).

However, some publications have contradicted the assumption of elevated FAAH and MAGL levels as tumor-promoting functional hallmarks. Accordingly, high levels of both enzymes were reported to positively correlate with the prognosis in terms of pancreatic ductal adenocarcinomas (Michalski et al., 2008).

In contrast to endocannabinoid-degrading enzymes, data on the expression levels of endocannabinoid-synthesizing enzymes are rare. In one report, evidence was provided for lower expression levels of *N*-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD), the main enzyme for AEA synthesis, in gliomas vs nontumor controls

(Wu et al., 2012). The same study further found FAAH and MAGL to be downregulated in glioma tissue, while the expression of the 2-AG-synthesizing enzyme DAG lipase remained unchanged, resulting in a decrease of AEA and an increase of 2-AG, respectively. Another investigation reported elevated messenger RNA (mRNA) levels of NAPE-PLD as well as of FAAH in colorectal cancer tissue (Chen et al., 2015). Finally, a downregulation of MAGL was found in endometrial carcinoma (Guida et al., 2010).

Taken together, the data concerning the regulation of endocannabinoids and their degrading enzymes cannot be considered as reliable tumor markers and do not provide consistent information regarding their functional role in tumor progression.

2.3 Cannabinoid Receptor Expression in Cancerous Lesions

Several studies addressing the regulation of cannabinoid receptors in cancerous lesions reported higher cannabinoid receptor expression levels in cancer cells or tissues as compared to healthy counterparts. Accordingly, an early investigation revealed CB_2 receptor upregulation to be directly related to tumor malignancy (Sanchez et al., 2001). Later, the CB_2 receptor was shown to be expressed in a high percentage of human acute myeloid leukemias (Alberich Jorda et al., 2004) and to be elevated in breast cancer (Caffarel, Sarrio, Palacios, Guzman, & Sanchez, 2006), in endometrial carcinoma (Guida et al., 2010), and in bladder tumors (Bettiga et al., 2017). Recent investigations reported a correlation between high levels of CB_2 receptor expression and reduction of disease-specific survival of patients suffering from head and neck squamous cell carcinoma (Klein Nulent et al., 2013) and human epidermal growth factor receptor 2 (HER2)-positive breast cancer (Pérez-Gómez et al., 2015).

The CB_1 receptor was found to be upregulated in mantle cell lymphoma compared to reactive lymphoid tissue (Islam et al., 2003). An increased CB_1 receptor expression has been shown to be associated with poor prognosis in pancreatic cancer (Michalski et al., 2008) and with disease severity and worse outcome in prostate cancer (Chung et al., 2009). The latter finding was substantiated by investigations showing CB_1 upregulation to correlate with Gleason score in prostate cancer (Fowler, 2015). CB_1 receptor expression levels were furthermore demonstrated as marker for adverse outcome in ovarian cancer (Messalli, Grauso, Luise, Angelini, & Rossiello, 2014) as well as advanced stage colorectal cancer (Jung et al., 2013). In human

hepatocellular carcinomas, CB_1 receptors were recently reported to appear increased relative to tumor-free areas (Mukhopadhyay et al., 2015). In contrast, another study indicated CB_1 receptor downregulation in clear cell renal carcinoma (Larrinaga et al., 2010). In agreement with this finding, analyses of mRNA obtained from human grade II–III colon carcinomas revealed a downregulation of the CB_1 receptor in 18 of 19 cancer specimens as compared to adjacent healthy mucosa, whereas the CB_2 receptor here remained virtually unaltered (Wang, Wang, Ning, Backlund, Dey, & DuBois, 2008).

Concerning an implication of cannabinoid receptors in hepatocellular carcinoma, overexpression of both cannabinoid receptors was found to be associated with improved prognosis (Xu et al., 2006). On the other hand, a correlation between cannabinoid receptor levels and malignancy was not found in glioblastoma patients (Held-Feindt, Dorner, Sahan, Mehdorn, & Mentlein, 2006).

Collectively, cannabinoid receptor upregulation seems to exhibit a tendency to adverse outcome in terms of some cancer entities. However, cannabinoid receptors do not serve as reliable markers or predictor for cancer development.

3. THE ROLE OF THE ENDOCANNABINOID SYSTEM IN CANCER PROGRESSION AND POTENTIAL PHARMACOLOGICAL OPTIONS OF CANNABINOID COMPOUNDS

The currently available data concerning the effects of the endocannabinoid system on cancer progression provide promising results that suggest cannabinoids as potential antineoplastic agents. With reference to the mechanisms of action, a modulation of several factors has been reported to be associated with suppression of angiogenesis and tumor cell invasion. These facts will be discussed in the following sections.

3.1 Cannabinoids as Anticancer Drugs

Despite the lack of comprehensive clinical data, a large number of in vitro and in vivo studies found cannabinoids to elicit a broad array of anticancer effects with anticarcinogenic effects on cancers of different origin. Probably most comprehensively investigated are effects of cannabinoids

on glioblastoma, initiated by experiments that revealed a tumor-regressive action of THC and WIN 55,212-2 to be associated with cannabinoid receptor activation and intracellular ceramide accumulation (Galve-Roperh et al., 2000). Other tumor entities for which cannabinoids were preclinically demonstrated as effective antineoplastic agents include pheochromocytomas (Sarker, Obara, Nakata, Kitajima, & Maruyama, 2000), thyroid epitheliomas (Bifulco et al., 2001), skin carcinomas (Casanova et al., 2002, 2003; Glodde, Jakobs, Bald, Tüting, & Gaffal, 2015), prostate cancers (Nithipatikom et al., 2004), leukemias (McKallip et al., 2006), mantle cell lymphomas (Gustafsson, Christensson, Sander, & Flygare, 2006), pancreatic cancers (Carracedo et al., 2006; Fogli et al., 2006), breast cancers (McAllister et al., 2011; Qamri et al., 2009), rhabdomyosarcomas (Oesch et al., 2009), cervical cancers (Lukhele & Motadi, 2016; Ramer & Hinz, 2008), cholangiocarcinomas (Leelawat, Leelawat, Narong, & Matangkasombut, 2010), colon cancers (Patsos et al., 2010), gastric cancers (Ortega et al., 2016; Xian et al., 2010), neuroblastomas (Hamtiaux et al., 2011), nonsmall cell lung cancers (Ramer et al., 2013), hepatocarcinomas (Pourkhalili et al., 2013), head and neck squamous cell carcinomas (Park et al., 2015), bladder carcinomas (Bettiga et al., 2017; Gasperi et al., 2015), and multiple myeloma (Barbado et al., 2017).

The spectrum of anticarcinogenic impacts of cannabinoids encompass antiproliferative effects (De Petrocellis et al., 1998) as well as induction of apoptosis (Galve-Roperh et al., 2000) and autophagy (Hernández-Tiedra et al., 2016; Salazar et al., 2009; Shrivastava, Kuzontkoski, Groopman, & Prasad, 2011; Vara et al., 2011). Additionally, cannabinoids were shown to enhance the tumor-immune surveillance system as shown in Fig. 1A (Haustein et al., 2014; Kishimoto et al., 2005). Some studies even suggested cannabinoids as possible adjuvants due to their virtue to reduce chemoresistance as has been shown for THC and CBD combined with vinblastine in leukemia cells (Holland et al., 2006) as well as for combinations of CBN, CBD, or THC with mitoxantrone in fibroblasts (Holland, Lau, Allen, & Arnold, 2007). In agreement with this notion, a recent report was able to demonstrate WIN 55,212-2 to support the antimyeloma activity of dexamethasone and melphalan thereby overcoming the resistance to melphalan in cell culture experiments (Barbado et al., 2017). Other studies found CBD to enhance the sensitivity of glioblastoma cells toward the chemotherapeutics carmustine, temozolomide, doxorubicin, and cisplatin (Deng, Ng, Ozawa, & Stella, 2017; Nabissi et al., 2013) as well as of multiple

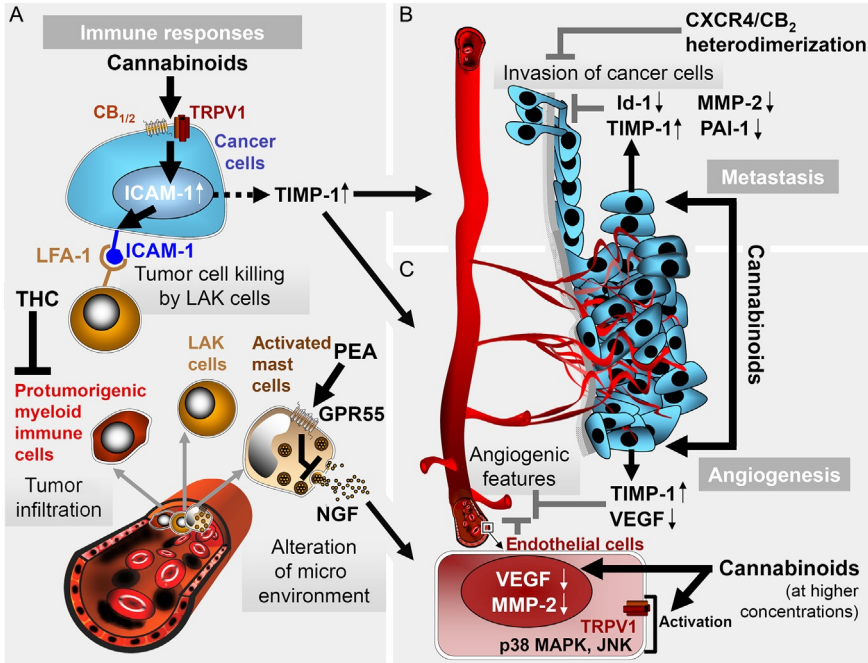

Fig. 1 Selected antineoplastic mechanisms by cannabinoids besides induction of cancer cell apoptosis. (A) Cannabinoids induce expression of intercellular adhesion molecule-1 (ICAM-1) in lung cancer cells via activation of the cannabinoid receptors CB_1 and CB_2 as well as the nonselective cation-channel transient receptor potential vanilloid 1 (TRPV1). ICAM-1 subsequently acts as counter receptor at the lymphocyte function-associated antigen-1 (LFA-1) on the surface of lymphokine-activated killer (LAK) cells conferring LAK cell-induced tumor cell killing. The endocannabinoid-like substance PEA inhibits the release of nerve growth factor (NGF) from mast cells via activation of the orphan receptor GPR55. Endothelial cells exposed to culture media of PEA-treated mast cells exhibit reduction of angiogenic features. ICAM-1 further acts as intracellular signaling molecule that induces enhanced release of tissue inhibitor of matrix metalloproteinases-1 (TIMP-1). THC inhibits the infiltration of protumorigenic myeloid immune cells into skin cancer tissue thereby conferring a less fruitful microenvironment for cancer cell growth. (B) Cannabinoid-induced TIMP-1 release inhibits lung and cervical cancer cell invasion. Further antiinvasive mechanisms of cannabinoids include decrease of matrix metalloproteinase-2 (MMP-2; gastric, breast cancer, glioblastomas), plasminogen activator inhibitor-1 (PAI-1; lung cancer) as well as reduction of inhibitor of DNA binding-1 (Id-1; glioblastomas, breast cancer). Furthermore, a $CXCR4/CB_2$ receptor heterodimerization was found to inhibit breast cancer cell invasion induced by the CXCR4 chemokine receptor ligand, stromal cell-derived factor-1α (SDF1α). (C) Inhibition of tumor neovascularization by cannabinoids is associated with decrease of vascular endothelial growth factor (VEGF) in experimental glioblastomas, skin, thyroid, and lung cancers. Conditioned media from lung cancer cells challenged with cannabinoids contain increased amounts of TIMP-1 that cause inhibition of angiogenic capacities of endothelial cells. Higher concentrations of cannabinoids that directly inhibit angiogenic features of endothelial cells such as proliferation and migration are associated with downregulation of VEGF, MMP-2, as well as activation of TRPV1, c-Jun NH_2-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK).

myeloma cells toward the proteasome inhibitor bortezomib via activation of TRPV2 (Morelli et al., 2014). Furthermore, a CBD/THC combination was shown to synergistically increase cell death and inhibition of migration induced upon treatment of multiple myeloma cells with the proteasome inhibitor carfilzomib (Nabissi et al., 2016). THC alone has further been demonstrated to support the apoptotic impact of the cytotoxic drugs cytarabine, doxorubicin, and vincristine on leukemia cells via downregulation of p42/44 MAPK (Liu, Scott, Shamash, Joel, & Powles, 2008). In addition, the cannabinoid compounds arachidonoyl cyclopropylpamide (ACPA, CB_1 agonist) and GW405833 (CB_2 agonist) have been reported to enhance chemosensitivity of pancreatic cancer cells toward gemcitabine by an enhanced ROS-dependent induction of autophagy (Donadelli et al., 2011). A synergistic proapoptotic action was further described for the combination of AEA and paclitaxel when using gastric cancer cells (Miyato et al., 2009).

Meanwhile there are several studies that provide evidence for a synergistic action of CB_1 receptor antagonists with chemotherapeutics. As such, one investigation reported an antiproliferative synergism on pancreatic cancer cells between gemcitabine and the CB_1 receptor antagonist SR141716 via enhanced ROS production and autophagy. In this study, SR141716 and gemcitabine further caused a drastic growth inhibition of pancreatic cancer xenograft (Donadelli et al., 2011). Interestingly, the CB_1 antagonist AM-251 has also been shown to gain the toxic impact of 5-fluorouracil on pancreatic cancer cells (Fogli et al., 2006). On the basis of gene expression profile analyses, the authors of the latter study assumed modulation of JAK/STAT and MAPK signaling network as underlying mechanism. This synergism was here discussed as a result of AM-251's diarylpyrazole structure exhibiting substantial structural similarity to the selective COX-2 inhibitor celecoxib rather than specific modulation of the endocannabinoid system. In line with this notion, AM-251 has been demonstrated to induce a synergistic antitumor activity on melanoma cells when combined with celecoxib (Carpi et al., 2015).

An overview of preclinical data on combinations of cannabinoids and chemotherapeutics is provided in Table 1. Here, results from studies that evaluated a synergistic action of cannabinoids combined with chemotherapeutics on the viability of cancer cells were included.

Finally, a recent study observed the combinational treatment of THC/CBD to even enhance the radiosensitivity of cancer cells in an orthotopic murine glioma model (Scott, Dalgleish, & Liu, 2014).

Table 1 Cannabinoids That Enhance Chemosensitivity in Combination With Currently Used Anticancer Drugs

Cannabinoid	Anticancer Drug	Mechanism	Cell Type	Reference
ACPA GW405833 SR141716	Gemcitabine	Autophagy ↑ ROS ↑ NF-κB ↑	Pancreatic cancer	Donadelli et al. (2011)
AEA	Paclitaxel	Caspase-3, -8, and -9 ↑	Gastric cancer	Miyato et al. (2009)
AM-251	Celecoxib	n.d.	Melanoma	Carpi et al. (2015)
	5-Fluorouracil	n.d.	Pancreatic cancer	Fogli et al. (2006)
CBD	Bortezomib	TRPV2 activation	Multiple myeloma	Morelli et al. (2014)
	Carmustine Doxorubicin Temozolomide	TRPV2 activation	Glioblastoma	Nabissi et al. (2013)
	Carmustine Cisplatin Temozolomide	n.d.	Glioblastoma	Deng et al. (2017)
CBD/THC	Carfilzomib	Adducts with the β5i subunit ↑	Multiple myeloma	Nabissi et al. (2016)
CBD THC	Vinblastine	p-GP expression ↓	Leukemia	Holland et al. (2006)
CBD CBN THC	Mitoxantrone	ABCG2 inhibition	Murine embryonic fibroblast	Holland et al. (2007)
THC	Cytarabine Doxorubicin Vincristine	p42/44 MAPK ↓	Leukemia	Liu et al. (2008)
WIN 55,212-2	Dexamethasone Melphalan	n.d.	Multiple myeloma	Barbado et al. (2017)

Arrows (third column) specify cannabinoid-induced upregulation (↑) or downregulation (↓) of the indicated intracellular parameter/mechanism involved in the toxic effects that result from combination of the respective cannabinoid (first column) with the anticancer drug (second column). The fourth column specifies the cell types affected by the combination indicated in the respective line. *Abbreviations*: *ABCG2*, ATP-binding cassette, subfamily G2; *ACPA*, arachidonoyl cyclopropamide; *AEA*, *N*-arachidonoylethanolamine, anandamide; *CBD*, cannabidiol; *CBN*, cannabinol; *GW405833*, CB_2 agonist; *MAPK*, mitogen-activated protein kinase; *n.d.*, not determined; *p-GP*, p-glycoprotein; *ROS*, reactive oxygen species; *SR141716*, CB_1 antagonist; *THC*, Δ^9-tetrahydrocannabinol; *TRPV2*, transient receptor potential vanilloid 2; *WIN 55,212-2*, potent non-selective cannabinoid receptor agonist; *β5i subunit*, catalytic subunit of the proteasome.

3.2 Effects of Cannabinoids on Tumor Angiogenesis

3.2.1 In Vivo Effects of Cannabinoids on Tumor Angiogenesis

The knowledge obtained from animal experiments using immunodeficient mice suggests cannabinoids to inhibit neovascularization in several different cancer types. Accordingly, inhibition of tumor angiogenesis has been reported for epidermal tumors (Casanova et al., 2003), melanomas (Blazquez et al., 2006), breast (Caffarel et al., 2010; Qamri et al., 2009), and lung cancer (Preet et al., 2011) when mice were treated with WIN 55,212-2 or JWH-133. JWH-133 was further demonstrated to be effective against neovascularization of glioma xenografts (Blazquez et al., 2003). Additionally, THC was found to inhibit tumor angiogenesis in models of lung (Preet, Ganju, & Groopman, 2008) and breast cancer (Caffarel et al., 2010) as well as in glioblastomas (Hernán Pérez de la et al., 2013). Furthermore, CBD was proven to inhibit tumor angiogenesis in experimental lung cancer tissue (Ramer et al., 2013). HU-311, a chinone derivative of CBD with topoisomerase II inhibitory properties, was successfully tested for antiangiogenic action in colon carcinomas (Kogan et al., 2006).

Although the antiangiogenic effects of cannabinoids in murine tumor xenograft systems appear undoubtful, the general mechanisms by which cannabinoids elicit their antiangiogenic impact are still a matter of debate. The following parts will address the different cellular aspects of cannabinoids' impact on angiogenesis.

3.2.2 Inhibition of Angiogenic Capacities of Endothelial Cells by Cannabinoids

Several studies revealed cannabinoids to directly inhibit angiogenic capacities of endothelial cells. Thus, the cannabinoids WIN 55,212-2 and JWH-133 were found to suppress migration and survival of human umbilical vein endothelial cells (HUVEC) associated with decrease of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-2 (MMP-2) expression (Blazquez et al., 2003). Another investigation addressing a probable involvement of endocannabinoids in endothelial injuries and shock conditions revealed AEA to exhibit toxic effects toward HUVEC via TRPV1 (Yamaji et al., 2003). As pivotal events conferring this toxic impact, phoshorylations of p38 mitogen-activated protein kinase (MAPK) and c-Jun NH_2-terminal kinase (JNK) were identified. Later, the stable AEA analog, 2-methyl-2′-F-anandamide (Met-F-AEA), was found to inhibit basic fibroblast growth factor-induced proliferation of pig aortic endothelial cells and HUVEC associated with decreased MMP-2 expression (Pisanti et al., 2007). The latter

study further revealed Met-F-AEA to inhibit vascular formation in the chick chorioallantoic membrane assays. Furthermore, the CBD chinone HU-331 was found to directly inhibit angiogenic features of endothelial cells (Kogan et al., 2006). Additionally, CBD has been attributed to suppress angiogenic features of endothelial cells (Solinas et al., 2012). Further cannabinoid compounds that were found to act antiangiogenically toward endothelial cells are the hexahydrocannabinol analogs LYR-7 and LYR-8 (Thapa et al., 2011), HU-210, and AEA (Rajesh et al., 2010).

However, some contradictory results have been reported concerning effects of cannabinoids on several types of endothelial cells. Accordingly, knockdown of the CB_1 receptor or pharmacological inhibition of CB_1 was associated with decreased migration, proliferation, and tube formation of growth factor-challenged HUVEC (Pisanti et al., 2011). In agreement with this notion, AEA was here found to even enhance angiogenic capacities. Notably, in other reports AEA was observed to act proapoptotic on the same type of endothelial cells (Yamaji et al., 2003). A simple explanation for this discrepancy may be given by the different concentrations used in the respective studies. Whereas higher concentrations of AEA (10 μM) elicit apoptosis and inhibition of endothelial activation (Yamaji et al., 2003), lower submicromolar concentrations of AEA (0.1 nM–0.1 μM) induce angiogenic features of endothelial cells (Pisanti et al., 2011). Noteworthy, *N*-arachidonoyl serine, another arachidonic acid derivative implicated in endocannabinoid-like action, was shown to elicit proangiogenic effects via activation of GPR55 (Zhang, Maor, Wang, Kunos, & Groopman, 2010). However, a probable proangiogenic impact restricted to the group of endocannabinoids at lower concentrations seems unlikely due to recent findings demonstrating modest proangiogenic effects when HUVEC were directly exposed to 3 μM concentrations of either CBD, THC, R(+)-methanandamide, or JWH-133 (Ramer et al., 2014). Taken into further account an earlier report demonstrating THC and CBD to confer proangiogenic effects when tested at a concentration of 50 nM (Kogan et al., 2006), it is tempting to speculate that cannabinoids may act in a proangiogenic manner toward endothelial cells at pharmacologically relevant concentrations. Accordingly, an analysis of plasma concentrations in patients treated with CBD yielded plasma peaks of 36 nM (Consroe, Kennedy, & Schram, 1991). Moreover, oral doses of 15 and 20 mg THC resulted in average peak plasma concentrations of 30 and 46 nM, respectively (Wall, Sadler, Brine, Taylor, & Perez-Reyes, 1983). Thus, these in vitro findings partly contradict the large number of preclinical in vivo findings

demonstrating inhibition of angiogenesis in cancer tissue by cannabinoids. The antiangiogenic actions of cannabinoids tested at higher concentrations are depicted in Fig. 1C.

3.2.3 Suppression of Tumor Angiogenesis by Cannabinoid-Modulated Intercellular Cross Talks

Taken into further consideration various factors modulated by cannabinoids in cancer or immune cells, cannabinoid-induced anti-angiogenesis in cancer tissue may likewise arise from tumor-to-endothelial or immune-to-endothelial cell communications finally leading to reduced neovascularization.

As depicted in Fig. 1C, diverse cannabinoids (i.e., CBD, THC, R(+)-methanandamide, and JWH-133) were demonstrated to alter the microenvironment of lung cancer cells thereby conferring inhibition of angiogenic capacities of endothelial cells. As mechanism of action, the aforementioned cannabinoids were demonstrated to inhibit angiogenesis via stimulating the release of tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) from cancer cells (Ramer et al., 2014). TIMP-1 acting as an endogenous inhibitor of MMPs exhibits antiangiogenic effects (Oh et al., 2004), although some data likewise support an MMP-independent effect on endothelial cells (Akahane, Akahane, Shah, Connor, & Thorgeirsson, 2004). The assumption of a cannabinoid-induced tumor-to-endothelial communication resulting in attenuation of cancer-related angiogenesis is further substantiated by a study that found conditioned media from AEA-treated breast cancer cells to inhibit endothelial cell proliferation (Picardi, Ciaglia, Proto, & Pisanti, 2014). In this study, inhibition of HUVEC proliferation was associated with downregulation of several angiogenesis-related factors such as VEGF, leptin, interferon-γ, and thrombopoietin. Other studies have suggested a modulation of inflammatory immune cells to contribute to cannabinoids' antiangiogenic responses. Accordingly, the endocannabinoid-like substance PEA was found to reduce the release of nerve growth factor (NGF) into conditioned media from activated mast cells via GPR55 thereby eliciting inhibition of endothelial cell proliferation (Fig. 1A) (Cantarella et al., 2011). Concerning further effects of cannabinoids on immune responses, a recent study reported THC to decrease the number of inflammatory immune cells in the microenvironment of experimental melanomas (Glodde et al., 2015). Interestingly, in this investigation the authors did not observe a toxic impact of THC on skin cancer cells in vitro. Nevertheless, THC was found to inhibit the recruitment of

protumorigenic myeloid immune cells in vivo thereby producing a less fruitful microenvironment for cancer cell growth without alteration of tumor angiogenesis (Fig. 1A).

The main factors expected to be modulated in an intercellular communication resulting in antiangiogenic effects of cannabinoids include growth factors such as VEGF as well as members of the MMP family. In fact, a downregulation of MMP-2 by several cannabinoid compounds in different tumor types was found to be associated with the antiangiogenic and antiinvasive action of the respective substances (Blazquez et al., 2003; Caffarel et al., 2010; Ramer & Hinz, 2008; see also Section 3.3). Using murine glioma and human astrocytoma xenograft models, an early investigation reported JWH-133 to decrease the levels of VEGF and MMP-2 (Blazquez et al., 2003). Later, the authors found JWH-133 to downregulate the upstream mediator of VEGF expression, hypoxia-inducible factor-1α on the transcriptional level (Blázquez et al., 2004). A cannabinoid-induced downregulation of VEGF was also confirmed for experimental skin tumors following treatment with the cannabinoids JWH-133 and WIN 55,212-2 (Casanova et al., 2003), for rat thyroid cancer cells exposed to Met-F-AEA (Portella et al., 2003), as well as for lung cancer cells treated with THC (Preet et al., 2008).

The complex interaction of different cell types involved in angiogenic responses affected by cannabinoid compounds is illustrated in Fig. 1A and C.

3.3 Effects of Cannabinoids on Metastasis

A number of reports found cannabinoids to elicit antiinvasive effects in vitro as well as a reduction of metastasis in rodent models. In an early work published in this field, 2-AG was demonstrated to suppress invasion of prostate cancer cells in a CB_1-dependent manner (Nithipatikom et al., 2004). As intracellular antiinvasive mechanisms, cannabinoids were found to downregulate inhibitors of DNA binding (Ids), among which Id-1 was described to support progression of metastasis of breast cancer cells into the lung by inhibiting basic helix-loop-helix transcription factors (Minn et al., 2005). In this context, the decrease of breast cancer cell invasion by CBD was associated with downregulation of Id-1 (McAllister, Christian, Horowitz, Garcia, & Desprez, 2007). An Id-1-dependent antiinvasive property of CBD was later substantiated by experiments using brain cancer as well as primary glioblastoma cells (Soroceanu et al., 2013). Recently, O-1663, a bicyclic resorcinol with selective affinity to the CB_2 receptor, was

discovered as another cannabinoid with antiinvasive properties dependent on Id-1 inhibition (Murase et al., 2014).

As further important metastasis-related factors that facilitate cancer spreading by virtue of their proteolytic action but also independent thereof (for review, see Ramer & Hinz, 2016), members of the MMP family have been described to be modulated by cannabinoids on the level of expression (Curran & Murray, 2000; Stamenkovic, 2000). In this context, MMP-2 has been shown to be downregulated by cannabinoid compounds in several reports. As such, the selective CB_2 agonist JWH-133 conferred MMP-2 decrease in glioma cells in vitro and in xenograft tissue (Blazquez et al., 2003). MMP-2 downregulation was further associated with decreased invasion of glioma cells challenged with THC (Blázquez et al., 2008), of cervical cancer cells treated with R(+)-methanandamide or THC (Ramer & Hinz, 2008), of gastric cancer cells treated with WIN 55,212-2 (Xian et al., 2010), and of hepatocarcinoma cells treated with the CB_1 receptor agonist arachidonoyl 2′-chloroethylamide or the high affinity and selective CB_2 agonist CB65, respectively (Pourkhalili et al., 2013). The antiinvasive and MMP-2-downregulating properties of arachidonoyl 2′-chloroethylamide and R(+)-methanandamide were further substantiated in experiments using breast cancer cells (Farsandaj, Ghahremani, & Ostad, 2012). Remarkably and in contrast to the observed antiinvasive and MMP-2-attenuating effects caused by CB65 in liver cancer cells (Pourkhalili et al., 2013), the authors here found CB65 to increase both invasiveness and MMP-2 expression of breast cancer cells. Noteworthy in this context, in another study CBD did not alter MMP-2 expression in cervical cancer cells (Ramer, Merkord, et al., 2010). Thus, MMP-2 downregulation by cannabinoids may appear in a cancer cell type-dependent manner.

Considering further members of the MMP family modulated upon cannabinoid treatment, the regulation of the endogenous inhibitor of MMP, TIMP-1, was focused on as key regulator of cannabinoid-modulated cancer cell invasion in past years. In previous studies, TIMP-1 was shown to negatively correlate with metastatic promotion in athymic mice (Khokha et al., 1989) and to exhibit an antiinvasive impact on cancer cells in vitro (Cattaneo, Fontanella, Canton, Delia, & Biunno, 2005; Ramer, Eichele, & Hinz, 2007). TIMP-1 becomes induced by THC, R(+)-methanandamide and CBD in cervical and lung cancer cells (Ramer & Hinz, 2008; Ramer, Merkord, et al., 2010; Ramer et al., 2012). Using small interfering (si) RNA approaches, evidence was provided to suggest a causal relation between TIMP-1 upregulation and the antiinvasive properties of

THC and R(+)-methanandamide (Ramer & Hinz, 2008) as well as CBD (Ramer, Merkord, et al., 2010; Ramer et al., 2012). The intercellular adhesion molecule-1 (ICAM-1) was later identified as upstream regulator of cannabinoid-induced TIMP-1 expression in lung cancer cells (Ramer et al., 2012). In addition, ICAM-1, upregulated upon treatment of lung cancer cells with diverse cannabinoids, has recently been further found to act as counter receptor for the lymphocyte function-associated antigen-1 (LFA-1) on the surface of lymphokine-activated killer cells (LAK), thereby conferring enhanced killing of cannabinoid-challenged cancer cells (Haustein et al., 2014). The diverse actions of ICAM-1 are presented in Fig. 1A and B.

A recent publication revealed the FAAH inhibitors URB597 and *N*-arachidonoyl serotonin (AA-5HT) as well as the FAAH substrates AEA and OEA to likewise block lung cancer cell invasion by virtue of their TIMP-1-inducing properties (Winkler et al., 2016). Moreover, a contribution of 2-AG turnover to tumor cell invasion was shown by findings indicating the MAGL inhibitor JZL184 and small interfering RNA (siRNA) targeting MAGL to inhibit invasion of colorectal cancer cell lines associated with downregulation of cyclin D1 and B-cell lymphoma 2 (Bcl-2) (Ye et al., 2011).

Searching for further antiinvasive mechanisms of CBD, plasminogen activator inhibitor-1 (PAI-1) was found to be downregulated in CBD-treated lung cancer cells. In this study, CBD's antiinvasive properties were partly reversed in the presence of recombinant PAI-1 at concentrations not eliciting a basal proinvasive action. Thus, a suppression of PAI-1 may contribute, at least in part, to the antiinvasive action of CBD (Ramer, Rohde, Merkord, Rohde, & Hinz, 2010).

THC has been found to modulate several downstream targets of epidermal growth factor (EGF) signaling such as Akt, p42/44 MAPK, and JNK resulting in decreased invasiveness of EGF-activated cancer cells (Preet et al., 2008). Antiinvasive effects were also reported for the selective CB_2 receptor agonists AM-1241 and JWH-015 with both compounds inhibiting the migration of breast cancer cells activated by the agonist at the chemokine receptor CXCR4, stromal cell-derived factor-1α (SDF1α). The authors postulated a CXCR4/CB_2 receptor heterodimerization to be responsible for reduced cancer cell migration as presented in Fig. 1B (Coke et al., 2016). An interference of cannabinoid compounds with CXCR4 signaling was likewise found in multiple myeloma cells (Nabissi et al., 2016). In this study, CBD, THC, a combination of both cannabinoids as well as a combination of both cannabinoids with carfilzomib were shown to inhibit SDF1α-induced migration of a multiple myeloma cell line. This study

additionally found treatment with CBD and THC combined with carfilzomib to inhibit chemotaxis of these cells challenged with eCyPA, an agonist at the CD147 receptor, that triggers myeloma bone marrow homing. The data concerning cannabinoids' antiinvasive mechanisms are presented in Fig. 1B.

In case of CBD acid, an enzymatic product of cannabigerolic acid, a downregulation of c-fos has been discussed as a further mechanism involved in inhibition of breast cancer cell migration (Takeda et al., 2017). Moreover, the antiinvasive action of Met-F-AEA was reported to include activation of the wingless/integrated (Wnt) signaling pathway via downregulation of Id-1. AEA-induced increase of glycogen synthase kinase 3β conferred subsequent decrease of β-catenin thereby inhibiting β-catenin-dependent oncogenes such as the markers of EMT, Snail1, Slug, and Twist (Laezza et al., 2013). The suppression of EMT by the endocannabinoid is presented in Fig. 2.

In vitro findings on antiinvasive properties of cannabinoids were confirmed in vivo by numerous reports using rodent models. Thus, inhibition of metastasis was observed in a mouse model of Lewis lung carcinoma metastasis in Met-F-AEA-treated animals (Portella et al., 2003). Additionally, WIN 55,212-2 was described to inhibit melanoma metastasis (Blazquez et al., 2006). In a murine cancer metastasis model, using a nonsmall cell lung cancer cell line, the antimetastatic impact of CBD was repeatedly confirmed (Ramer, Merkord, et al., 2010; Ramer et al., 2012). As underlying mechanism, the CBD-induced ICAM-1 expression by lung cancer cells could be proven as key signaling event. Accordingly, suppression of ICAM-1 by use of a neutralizing antibody partly reversed the inhibitory impact of CBD on lung cancer metastasis (Ramer et al., 2012). CBD has also been found to block breast cancer (McAllister et al., 2011) and glioblastoma metastasis (Soroceanu et al., 2013) with both effects being associated with downregulation of Id-1. The key role of Id-1 reduction within the antimetastatic mechanism of cannabinoids toward breast cancer was later also confirmed for the cannabinoid O-1663 (Murase et al., 2014). WIN 55,212-2 and JWH-133 were further approved as cannabinoid-based drugs conferring inhibition of breast cancer spreading in vivo (Qamri et al., 2009). Inhibition of lung cancer metastasis was also confirmed for THC (Preet et al., 2008). Finally, a recent investigation found the endocannabinoid-elevating FAAH inhibitors URB597 and AA-5HT to inhibit lung cancer metastasis in vivo (Winkler et al., 2016). In line with this data, the latter study likewise revealed antimetastatic properties for intraperitoneally applied AEA, 2-AG, OEA, and PEA.

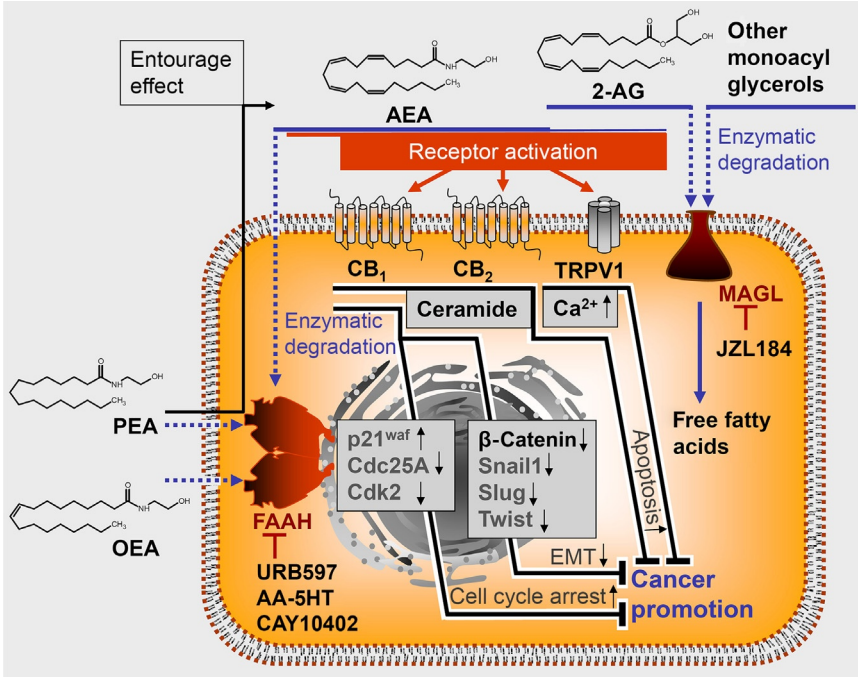

Fig. 2 Involvement of the endocannabinoid system in cancer progression. Fatty acid amide hydrolase (FAAH) confers hydrolysis of the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) as well as of the endocannabinoid-like substances oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) that do not exhibit affinity to cannabinoid receptors CB_1 and CB_2. Levels of AEA, 2-AG, OEA, and PEA are increased upon inhibition of FAAH by URB597, arachidonoyl-serotonin (AA-5HT), and CAY10402. PEA enhances effects of AEA at cannabinoid receptors via downregulation of FAAH and support of calcium (Ca^{2+}) conductance, assigned as entourage effect. Endocannabinoids in turn may inhibit cancer growth and induce apoptosis via cannabinoid receptor-triggered intracellular ceramide accumulation or TRPV1-dependent Ca^{2+} influx. AEA further inhibits cell cycle checkpoint response via upregulation of $p21^{waf}$, degradation of the dual specificity phosphatase Cdc25A converging on suppression of cyclin-dependent kinase 2 (Cdk2) activity. Transformation of cancer cells into invasive phenotypes is further suppressed by AEA via downregulation of the EMT markers β-catenin, Snail1, Slug, and Twist. Monoacylglycerol lipase (MAGL) is the major degrading enzyme for 2-AG but also for other monoacylglycerols. Inhibition of MAGL by JZL184 suppresses cancer growth via prolonged activation of cannabinoid receptors by 2-AG or by decrease of cancer-promoting free fatty acids. Factors written in *gray font* indicate transcriptionally modulated genes.

4. INHIBITION OF ENDOCANNABINOID-DEGRADING ENZYMES AS ANTICANCER STRATEGY

According to several research groups, the endocannabinoid system may pose an endogenous anticancer system (for review, see Petrosino & Di Marzo, 2010; Ramer & Hinz, 2016). This view is mainly based on findings that indicate endocannabinoids to act anticancerogenic and on experiments that clearly provide evidence for antineoplastic effects of drugs that inhibit endocannabinoid turnover.

With respect to anticancer properties of endocannabinoids, AEA was reported to inhibit proliferation of NGF- (De Petrocellis et al., 1998) and EGF-stimulated prostate cancer cells (Mimeault, Pommery, Wattez, Bailly, & Hénichart, 2003) as well as of mantle cell lymphoma cell lines (Gustafsson et al., 2006) and thyroid cancer cells (Bifulco et al., 2004). Antiproliferative effects of AEA and 2-AG were further confirmed in murine glioma (Jacobsson, Wallin, & Fowler, 2001) and colorectal cancer cells (Ligresti et al., 2003). As mechanism of action, AEA was found to modulate cell cycle arrest via upregulation of $p21^{waf}$, degradation of Cdc25A, and inhibition of the cyclin *E*–Cdk2 kinase complex activation in a CB_1 receptor-dependent manner (Fig. 2) (Laezza, Pisanti, Crescenzi, & Bifulco, 2006). Furthermore, AEA was found to elicit apoptosis of human gastric adenocarcinoma (Ortega et al., 2016), prostate cancer (Orellana-Serradell et al., 2015), colorectal cancer (Patsos et al., 2010), and melanoma cells (Adinolfi et al., 2013).

In agreement with the data obtained with AEA, inhibition of the AEA-degrading enzyme, FAAH, revealed similar anticancer effects (Ligresti et al., 2003). Most of these experiments were performed using the FAAH inhibitors AA-5HT (Bisogno et al., 1998) and URB597 (Alexander & Cravatt, 2005). An antiproliferative effect of AA-5HT was confirmed in thyroid cancer cells in vitro and in vivo (Bifulco et al., 2004). Noteworthy, antiproliferative effects of AA-5HT and AEA observed in the latter investigation were shown to involve a CB_1-dependent pathway. On the other hand, AA-5HT was found to completely reverse the formation of aberrant crypt foci regardless of the genotype (CB_1 knockout vs wild-type mice) in a rodent model with azoxymethane-induced colonic aberrant crypt foci (Izzo et al., 2008).

Using neuroblastoma cells, AEA was proven to elicit an antiproliferative action that became even enhanced in the presence of URB597 (Hamtiaux

et al., 2011). Similar results were obtained from experiments that addressed the effect of combinational treatment of inhibitors of endocannabinoid-degrading enzymes and PEA in melanoma cells (Hamtiaux et al., 2012). In this study, the FAAH inhibitors URB597 and CAY10402 as well as the unspecific FAAH/MAGL inhibitors MAFP and CAY10499 potentiated the cytotoxic impact of PEA toward melanoma cells. By use of a melanoma xenograft model, the latter study could further confirm a growth-inhibitory action of URB597 when combined with PEA, but not when administered alone. In contrast to the cytotoxic action of PEA shown by Hamtiaux et al. (2012), earlier reports found PEA to act as an entourage agent that enhances the cytostatic impact of AEA without causing a cytotoxic action on cancer cells per se (Fig. 2) (Di Marzo et al., 2001). Furthermore, a combination of URB597 with Met-F-AEA was demonstrated to confer a growth-inhibitory action via interference with EGF signaling in vitro and in vivo (Ravi, Sneh, Shilo, Nasser, & Ganju, 2014). As likewise demonstrated for melanoma cells (Hamtiaux et al., 2012), URB597 was later found to lack a tumor growth-inhibitory action in xenografts derived from lung cancer cells (Ravi et al., 2014; Winkler et al., 2016).

An overview of the connection between FAAH inhibition, prolonged action of endocannabinoids at their cognate receptors and the subsequent growth-inhibitory action is presented in Fig. 2.

A further target of endocannabinoid turnover, the 2-AG-degrading MAGL, has attracted considerable scientific interest as modulator of cancer growth. Accordingly, a recent study found knockdown of MAGL and treatment with the MAGL inhibitor JZL184 to elicit suppression of proliferation and induction of apoptosis in colorectal cancer cells (Ye et al., 2011). The latter report provided first-time proof for a tumor-regressive action of MAGL siRNA and JZL184 in vivo using a colon cancer xenograft model. In agreement with the tumor-regressive impact of MAGL inhibition, the MAGL substrate 2-AG was demonstrated to inhibit cell invasion of androgen-independent prostate carcinoma cells in a CB_1 receptor-dependent manner (Nithipatikom et al., 2004). The latter study further found inhibition of 2-AG synthesis by the DAG lipase inhibitor RHC-80267 to increase cancer cell invasion, vice versa. Additionally, MAGL inhibition by a small hairpin RNA approach or by treatment with JZL184 was associated with decreased invasion in vitro as well as with a growth-inhibitory action toward prostate carcinoma xenografts (Nomura et al., 2011). Here, the authors presented a dual mechanism underlying the antiinvasive and growth-inhibitory action of MAGL inhibition that partially

involved CB_1 receptor activation. In addition to CB_1 receptor activation, free fatty acids conferred a partial suppression of this antiinvasive action. Therefore, these products of MAGL activity were supposed to act as procancerogenic lipid precursors for synthesis of lysophosphatidic acid and prostaglandins. This hypothesis was substantiated using breast, ovarian, and melanoma cancer cells (Nomura et al., 2010). MAGL was additionally described as integral component of the EMT and cancer cell stemness according to a large-scale transcriptional analysis that monitored gene expression signatures of aggressive vs nonaggressive cancer cells (Nomura et al., 2011). The abovementioned facts concerning the role of the endocannabinoid system in cancer progression are summarized in Fig. 2.

5. CONCLUSION

When almost four decades ago Harris et al. (1978) were presenting the first data suggesting cannabinoids to stereospecifically bind to cell fractions, they stated that it remained to be determined "whether such binding has any relevance to the pharmacological effects of this interesting class of drugs." From the current point of view, it has to be concluded that far above this assumption, cannabinoid receptor modulation represents an integral component of ubiquitous physiological processes and appears as attractive target for treatment of neoplastic diseases. However, at present, it still remains to be unraveled whether cannabinoids may reach any clinical relevance for systemic cancer treatment.

Despite many beneficial effects, several studies have raised doubts concerning anticancer effects of cannabinoids with findings suggesting cannabinoids to even support cancer growth and spreading. As such, early investigations found mitotic effects of THC in prostate cancer (Sánchez, Ruiz-Llorente, Sánchez, & Díaz-Laviada, 2003) and glioblastoma cells (Hart, Fischer, & Ullrich, 2004). Furthermore, R(+)-methanandamide and THC were shown to increase tumor growth in immunocompetent mice (Gardner, Zhu, Sharma, Tashkin, & Dubinett, 2003; Zhu et al., 2000). Moreover, high cannabinoid receptor levels were demonstrated to confer mitotic effects by coupling to prosurvival pathways in astrocytoma cells (Cudaback, Marrs, Moeller, & Stella, 2010). A recent investigation even reported CB_1 receptor knockout mice or wild-type mice treated with a peripheral CB_1 receptor antagonist to develop fewer and smaller tumors in a chemically induced hepatocellular carcinoma model (Mukhopadhyay et al., 2015). Another study showed knockdown of CB_2 receptors in

HER-overexpressing breast cancer cells to be associated with decreased growth of xenografts and metastasis (Pérez-Gómez et al., 2015). In agreement with these findings, the CB_1 receptor antagonist SR141716 was found to act antineoplastic in some studies (Ciaglia et al., 2015; Sarnataro et al., 2006). However, these reports are in contrast to the vast majority of preclinical data that support cannabinoids or substances that enhance the endocannabinoid levels as option for anticancer treatment.

Established cannabinoid effects that cancer patients may benefit from result from reduction of severe side effects of currently used chemotherapeutics. Accordingly, cannabinoids are currently already used in cancer patients to suppress emesis and nausea (Tramèr et al., 2001), and to relieve cancer pain (Khasabova et al., 2012). A previous meta-analysis revealed cannabinoids as considerable option for the treatment of cancer-related pain (Martin-Sanchez, Furukawa, Taylor, & Martin, 2009). Another recently published meta-analysis assessed 28 studies (including 1772 participants) that addressed cannabinoid effects on nausea and vomiting due to chemotherapy. Here, benefits of cannabinoid treatments were recorded vs comparator (mostly prochlorperazine) or placebo without yielding statistical significance (Whiting et al., 2015).

Additionally, several protective effects were proven for cannabinoid compounds suggesting, e.g., benefits for cancer patients with chemotherapy-induced peripheral neuropathy (Gingerich, Wadhwa, Lemanski, Krahn, & Daeninck, 2009). Cannabinoids were furthermore demonstrated to palliate adverse effects of the chemotherapeutic agent cisplatin. In this context, the CB_2 agonist LEI-101 was found to decrease the nephrotoxicity of cisplatin in a murine model (Mukhopadhyay et al., 2016). With respect to systemic anticancer effects of cannabinoids, their synergistic interaction with a number of cytostatic drugs as well as their property to suppress metastasis and tumor-associated neovascularization is of particular interest.

Concerning putative combinational treatments in cancer therapies, cannabinoids seem to elicit a synergistic increase of the antitumorigenic action of conventional chemotherapeutics, while counteracting some of their adverse effects. Indeed, several recently published studies demonstrated THC and CBD to boost the cytostatic effects of a considerable number of chemotherapeutic drugs (Table 1). With respect to some reports that demonstrated cannabinoids to alter transport capacities of chemotherapeutic drugs via inhibition of efflux transporters such as p-glycoprotein (p-GP) (Holland et al., 2006; Molnár et al., 2000; Nieri et al., 2006), however, possible adverse drug interactions have to be taken into consideration.

The significance of drugs that elicit inhibition of metastasis arises from the fact that almost all fatal progressions of malignant diseases are associated with metastasis. Unfortunately, specific options to counteract metastasis are currently barely available. Hence, there is an urgent need for novel therapeutic approaches that specifically target metastatic processes. Based on the preclinical findings summarized here, cannabinoids may serve as "antimetastatics" to improve the clinical prospects of treating advance stage cancer diseases. In addition, cannabinoids may provide an option as antiangiogenic drugs. In this context, various innovative pharmacotherapeutical approaches currently pursue this strategy such as an antibody against VEGF, bevacizumab, that has been proven to provide benefits in metastatic renal cell cancer (Yang et al., 2003), colorectal cancer (Hurwitz et al., 2004), non-small cell lung cancer (Sandler et al., 2006), and ovarian cancer (Perren et al., 2011). Small molecules targeting tumor angiogenesis currently used are sunitinib, sorafenib, and pazopanib (Iacovelli et al., 2014). Thus, cannabinoid compounds may offer an attractive small molecule supplement for antiangiogenic treatments of solid tumors.

Unfortunately, data concerning efficacy and safety of cannabinoids are currently not available from clinical studies that address systemic effects on cancer progression beyond palliative use. In this context, it is tempting to speculate that, according to anecdotal reports, high-dose applications are favorable (Abrams, 2016). Concerning the safety and efficacy of cannabinoids, the recently published largest multicenter observational study (1615 patients from 30 centers) specified nabiximols (Sativex™), a combination of THC and CBD, as an effective and safe treatment for patients with multiple sclerosis with moderate to severe spasticity (Patti et al., 2016).

According to such clinical evaluations, the major therapy-limiting unwanted side effect of cannabinoids lies in the psychoactive properties of compounds such as THC that exert high affinity to the CB_1 receptor and pass the blood–brain barrier. As considerable alternatives for systemic cancer treatment, nonpsychoactive cannabinoids such as CBD or JWH-133 have been demonstrated to exert remarkable anticancer properties with respect to the existing preclinical expertise. In this context, CBD was demonstrated to exert high safety even when applied chronically with high doses up to 1.5 g/day (Bergamaschi, Queiroz, Zuardi, & Crippa, 2011). Thus, the anticarcinogenic effects of cannabinoids are not obligatory associated with psychoactivity. This particular fact favors nonpsychoactive cannabinoids for systemic cancer therapies.

Taken together, cannabinoids may support the future armamentarium for treatment of cancer diseases beyond their palliative use, as inhibitors of cancer growth, as cytostatic boosters, as antimetastatics, and as inhibitors of tumor neovascularization, given that clinical studies will exceed case reports and will provide evidence for considerable systemic benefits.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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