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REVIEW ARTICLE

Anti-tumour actions of cannabinoids

Correspondence Professor Dr Burkhard Hinz, Institute of Pharmacology and Toxicology, Rostock University Medical Center, Schillingallee 70, D-18057 Rostock, Germany. E-mail: burkhard.hinz@med.uni-rostock.de

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Burkhard Hinz and Robert Ramer

Institute of Pharmacology and Toxicology, Rostock University Medical Center, Rostock, Germany

The endocannabinoid system has emerged as an important target for the treatment of many diverse diseases. In addition to the well-established palliative effects of cannabinoids in cancer therapy, phytocannabinoids, synthetic cannabinoid compounds and inhibitors of endocannabinoid degradation have attracted attention as possible systemic anticancer drugs. Results emerging from preclinical studies suggest cannabinoids elicit effects at different levels of cancer progression, including inhibition of proliferation, neovascularization, invasion and chemoresistance, induction of apoptosis and autophagy as well as enhancement of tumour immune surveillance. Although the clinical use of cannabinoid receptor ligands is limited by their psychoactivity, non-psychoactive compounds, such as cannabidiol, have gained attention due to preclinically established anticancer properties and a favourable risk-to-benefit profile. Thus, cannabinoids may complement the currently used collection of chemotherapeutic agents, as a broadly diversified option for cancer treatment, while counteracting some of their severe side effects.

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Abbreviations

2-AG, 2-arachidonoylglycerol; AA-5HT, *N*-arachidonoyl serotonin; AEA, *N*-arachidonoylethanolamine, arachidonoylethanolamide, anandamide; ATF, activating transcription factor; Bcl-2, B-cell lymphoma 2; CBD, cannabidiol; Cdc, cell division cycle; CDK, cyclin-dependent kinase; DAGL, DAG lipase; EMT, epithelial-to-mesenchymal transition; FAAH, fatty acid amide hydrolase; ICAM-1, intercellular adhesion molecule 1; Id, inhibitor of DNA binding; MAGL, monoacylglycerol lipase; Met-F-AEA, 2-methyl-2′-*F*-anandamide; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; NAPE-PLD, *N*-acyl-phosphatidylethanolamine-selective PLD; OEA, *N*-oleoylethanolamine, oleoylethanolamide; PEA, *N*-palmitoylethanolamine, palmitoylethanolamide; Rb, retinoblastoma-associated protein; Sox, sex-determining region Y-box; TRB, tribbles homologue; xCT, cystineglutamate transporter

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The endocannabinoid system

The components of the 'classical' endocannabinoid system have been intensively investigated and reviewed during the last decades. According to its initial definition, the endocannabinoid system comprised the *Pertussis* toxin sensitive, $G_{i/o}$-coupled, cannabinoid **CB_1** and **CB_2** receptors (Matsuda *et al.*, 1990; Munro *et al.*, 1993), as well as their endogenous ligands **N-arachidonoylethanolamine** (anandamide, AEA) and **2-arachidonoylglycerol** (2-AG) (Devane *et al.*, 1992; Mechoulam *et al.*, 1995).

The endocannabinoid system further covers other cannabinoid receptor ligands, such as **2-arachidonyl glyceryl ether** (noladin ether, 2-AGE) (Hanus *et al.*, 2001), **N-arachidonoyldopamine** (NADA) (Bisogno *et al.*, 2000) and **O-arachidonoylethanolamine** (virodhamine) (Porter *et al.*, 2002). In addition, the fatty acid amides **N-homo-γ-linolenylethanolamine** and **N-docosatetra-7,10,13,16-enoylethanolamine** were reported to exhibit cannabinoid receptor binding properties (Hanus *et al.*, 1993).

Synthesizing and degrading enzymes of AEA and 2-AG comprise a group of proteins that have been investigated intensively following the discovery of endocannabinoids (see Di Marzo, 2009). AEA and other *N*-acylethanolamines are endogenously synthesized from membrane phospholipids by the enzyme **N-acylphosphatidylethanolamine-PLD** (NAPE-PLD) and *via* alternative biosynthetic pathways. 2-AG can be generated *via* **phospholipase C** or by turnover of DAG *via* DAG lipase (DAGL) **α** and **β**. The intracellular degradation of endocannabinoids is catabolized by the serine hydrolase **fatty acid amide hydrolase** (FAAH) (Deutsch and Chin, 1993) and, in terms of 2-AG, by the **monoacylglycerol lipase** (MAGL) (Blankman *et al.*, 2007).

Following the discovery of the two cannabinoid receptors, the cation channel **TRPV1** has been described as an additional receptor, activated by AEA (Zygmunt *et al.*, 1999) and **cannabidiol** (CBD), a non-psychoactive phytocannabinoid (Bisogno *et al.*, 2001). Moreover, recent investigations revealed **TRPV2** channels to be involved in the modulation of cell fate by CBD (Nabissi *et al.*, 2013, 2015).

In addition to these ionotropic cannabinoid receptors, several GPCRs were deorphanized as cannabinoid-triggered targets. Thus, **GPR55** was antagonized by CBD and activated by a panel of cannabinoid compounds (Ryberg *et al.*, 2007). Accordingly, the synthetic cannabinoid GP55940 was found to activate GPR55, whereas **WIN 55,212-2** did not bind or activate GPR55. The latter study further reported the endocannabinoid-like substance **palmitoylethanolamide** (PEA), as well as 2-AG and virodhamine, to show a significantly more potent action through GPR55 than through either CB_1 or CB_2 receptors, whereas AEA was equally active on CB receptors and on GPR55.

Finally, several *N*-acylethanolamines, including AEA as well as the endocannabinoid-like substances PEA, **oleoylethanolamide** (OEA), **stearoylethanolamide** and linoleoylethanolamide, were revealed as activators of **PPARα** with OEA exerting the highest efficacy (Artmann *et al.*, 2008). Furthermore, recent investigations suggest cannabinoid compounds can enhance PPARγ expression and activation (Ramer *et al.*, 2013; Vara *et al.*, 2013).

The following sections focus on the different levels of anti-tumour effects of cannabinoids and the role of components of the endocannabinoid system in this process. An overview of selected pathways involved in mediating the anticancerogenic effect of cannabinoids is provided in Figure 1.

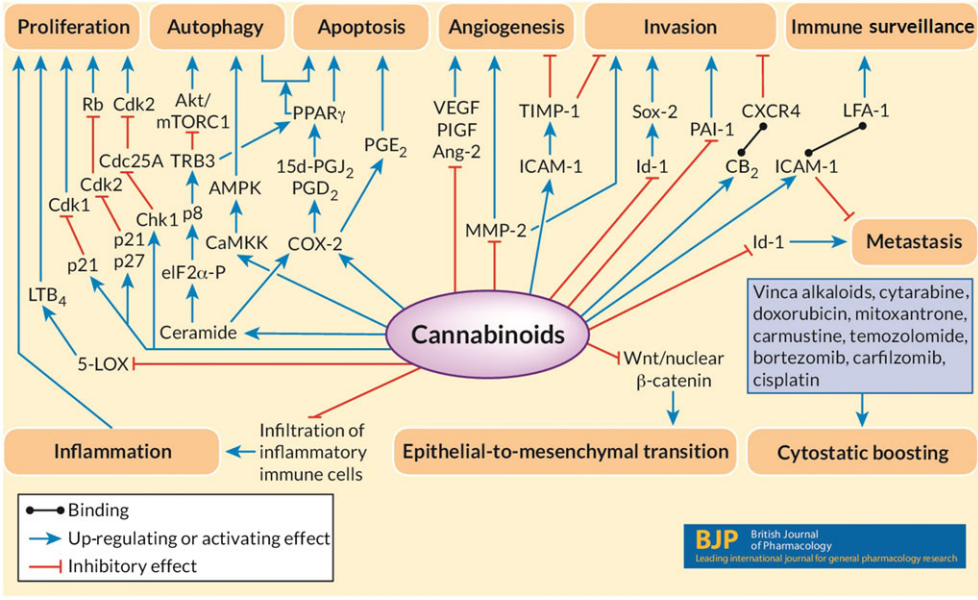

Figure 1

Selected pathways involved in anticancerogenic effects of cannabinoids. The diagram shows selected pro-cancerogenic and anti-cancerogenic pathways in cancer cells. Coloured arrows indicate inhibitory (red) and stimulatory (blue) effects of cannabinoids on these processes, finally leading to inhibition of cancer cell growth and spreading. Lines with dots at the end indicate binding between two factors.

Regulation of the endocannabinoid system in cancer tissues

A large number of investigations provided proof for up-regulation of components of the endocannabinoid system in malignant tissue and an association with adverse patient outcome. In this context, poor prognoses for cancer patients were associated with high expression of CB_1 receptors in malignant tissue, such as pancreatic cancer (Michalski *et al.*, 2008). Up-regulation was likewise observed for CB_2 receptors in a variety of cancer types (see Ramer and Hinz, 2017). Endogenous ligands at these receptors were similarly elevated, as has been reported for 2-AG, for example, in prostate cancer (Nithipatikom *et al.*, 2004). In human meningiomas, AEA, but not 2-AG, was up-regulated (Maccarrone *et al.*, 2001), whereas a converse regulation with decreased AEA and increased 2-AG was observed in blood analyses of circulating endocannabinoids from patients suffering from different kinds of cancers (Sailler *et al.*, 2014) as well as in glioma tissues (Wu *et al.*, 2012). The latter investigation further reported down-regulation of both expression and activity for NAPE-PLD, FAAH and MAGL, whereas the expression of DAGL remained unchanged. In colon tumour patients, MAGL expression was likewise down-regulated in tumour tissue, compared with the neighbouring healthy tissue and was either absent or reduced in the majority of primary colorectal cancer cases (Sun *et al.*, 2013). This study also reported reduced MAGL expression associated with lung, breast, stomach and ovary cancers. Analyses of biopsies obtained from prostate cancer patients further revealed higher expression levels of FAAH in cancer tissue (Endsley *et al.*, 2008). However, the association between levels of the endocannabinoid-degrading enzymes and prognosis for cancer patients was less clear-cut. Thus, high levels of FAAH and MAGL positively correlated with the prognosis of patients with pancreatic ductal adenocarcinomas (Michalski *et al.*, 2008), while an increase of MAGL expression was reported for highly malignant tissues, such as high-grade primary ovary tumours (Nomura *et al.*, 2010).

The functional implication of increased cannabinoid receptor expression and increased endocannabinoid levels in tumour tissue is currently a matter of active debate (see Ramer and Hinz, 2017). Moreover, components of the endocannabinoid system obviously do not possess useful uniform marker properties for reliable cancer prognosis. On the other hand, the overall view of the endocannabinoid system as an anticancer system is supported by numerous reports on anticancerogenic properties of cannabinoid compounds and inhibitors of endocannabinoid turnover, as discussed in the following sections.

Anti-tumour actions of cannabinoids

Tumour cell growth and viability

The first study monitoring the effects of phytocannabinoids on cancer regression in animal experiments was published by Munson *et al.* (1975), who showed suppression of tumour growth by Δ^8-tetrahydrocannabinol, **Δ^9-tetrahydrocannabinol** (THC) and **cannabinol**, long before the discovery of cannabinoid receptors and endocannabinoids. Meanwhile, cannabinoid compounds were revealed as potent inhibitors of cancer progression on different levels of cancer cell growth and spreading in numerous preclinical investigations.

During the last few decades, a large body of evidence has accumulated to suggest endocannabinoids, phytocannabinoids and synthetic cannabinoids exert an inhibitory effect on cancer growth *via* blockade of cell proliferation and induction of apoptosis. In the late 1990s, De Petrocellis *et al.* (1998), addressing the role of cannabinoid receptors in the growth inhibitory action of several cannabinoid compounds, reported AEA to reduce the proliferation of breast cancer cell lines at IC_{50} values of 0.5 μM (MCF-7 cells) and 1.5 μM (EFM-19 cells) *via* a CB_1 receptor-dependent mechanism. In their study, the synthetic cannabinoid **HU-210**, likewise, elicited a comparable antiproliferative action on EFM-19 cells but with less potency. Notably, although the authors did not mention the IC_{50} levels for the antiproliferative effects of HU-210, half maximal inhibition of proliferation can be estimated to be above 5 μM. Two years later, the first study demonstrating that THC and the synthetic cannabinoid WIN 55,212-2 caused regression of glioma growth in Wistar rats and in $Rag^{2-/-}$ mice was published (Galve-Roperh *et al.*, 2000). In that study, rats, treated with THC or WIN 55,212-2, were shown to live significantly longer than vehicle-treated animals. These experiments were supplemented by *in vitro* investigations showing that THC, as well as the synthetic cannabinoids WIN 55,212-2, **CP55940** and HU-210 inhibited glioma cell growth, associated with CB_1-dependent and CB_2-dependent sustained ceramide accumulation and p42/44 **MAPK** activation. Subsequently, the selective CB_2 receptor agonist **JWH-133** also inhibited growth of glioma cells (Sánchez *et al.*, 2001). Following these and other pioneering studies (see Caffarel *et al.*, 2012), a large number of investigations on anticancerogenic cannabinoid actions have been published.

In terms of the intracellular mechanisms of the antiproliferative actions of cannabinoids, several studies have demonstrated cannabinoid compounds to modulate cell cycle checkpoints. As such, cannabinoid receptor activation induced melanoma cell cycle arrest *via* inhibition of **Akt** and hypophosphorylation of the **retinoblastoma-associated protein** (Rb) (Blázquez *et al.*, 2006). While the latter and other studies reported cannabinoids to confer cell cycle arrest at the G_1–S transition, another study using breast cancer cells showed that THC arrested the G_2–M transition, *via* down-regulation of **cyclin-dependent kinase 1** [CDK1/cell division cycle (Cdc)2] and induction of p21, a CDK inhibitor that suppresses Cdc2–cyclin B activation (Caffarel *et al.*, 2006). A further investigation addressing the antiproliferative impact of a stable AEA analogue, 2-methyl-2′-*F*-anandamide (Met-F-AEA), on breast cancer cells, found a transient and delayed cell cycle checkpoint response (Laezza *et al.*, 2006). Accordingly, Met-F-AEA caused an increase of $p21^{waf}$ and $p27^{kip1}$ and a decrease of cyclins A and E. As upstream events of cyclin degradation, the authors demonstrated a rapid activation of **checkpoint kinase 1** that induced downstream degradation of Cdc 25 homologue A and **Cdk2** inactivation. As a delayed response, Met-F-AEA activated the $p21^{waf}$ cascade that additionally resulted in

Cdk2 inhibition. These effects were further associated with a reduction of Rb activity, a prominent target of Cdk2 activity.

As a mechanism of glioma cell death in response to THC treatment, a CB_2 receptor-mediated up-regulation of the stress-associated transcriptional co-activator p8 mediated a proapoptotic action *via* up-regulation of the endoplasmic reticulum stress-related genes activating transcription factor (ATF)-4 and the **pseudo-kinase tribbles homolog** (TRB)3 (Carracedo *et al.*, 2006). A contribution of p8 induction to cannabinoid-induced apoptosis was later substantiated for THC and HU-210 in rhabdomyosarcoma cells. In that study, cannabinoid-induced apoptosis was associated with inhibition of Akt signalling and, as shown for HU-210, restored by a CB_1 receptor antagonist (Oesch *et al.*, 2009). Another investigation reported THC and the selective CB_2 receptor agonist, JWH-133, to inhibit the growth of highly aggressive **ErbB2**-positive breast cancers, associated with inhibition of the pro-tumourigenic Akt pathway (Caffarel *et al.*, 2010).

A panel of investigations reported cannabinoid-induced impaired cancer cell viability *via* mechanisms bypassing activation of cannabinoid receptors. For example, CP55940, JW015 and the FAAH inhibitor, *N*-arachidonoyl serotonin (AA-5HT), inhibited proliferation of rat glioma cells independently of both CB receptors and TRPV1 channel activation (Jacobsson *et al.*, 2001). In the same investigation, however, AEA and 2-AG exerted antiproliferative actions *via* cannabinoid receptor-dependent and TRPV1-dependent oxidative stress and **calpain** activation. Furthermore, **R(+)-methanandamide** induced a cannabinoid receptor- and TRPV1-independent apoptosis in human neuroglioma cells by *de novo* synthesis of ceramide (Hinz *et al.*, 2004). In the latter type of cells, the proapoptotic mechanism of R(+)-methanandamide was based on a ceramide-dependent up-regulation of **COX**-2 expression (Ramer *et al.*, 2003) and increased synthesis of proapoptotic **PGE_2** (Hinz *et al.*, 2004).

Notably, reports on anticancer effects of CBD, a non-psychoactive cannabinoid with low affinity to CB receptors, revealed proapoptotic effects, without CB receptor activation. Thus, CBD suppressed proliferation of glioma cells *via* decreased activity and content of **5-lipoxygenase** and of its end product **LTB_4** (Massi *et al.*, 2008). Another study found CBD to induce PPARγ-dependent toxicity by upstream induction of COX-2-dependent PGs of the D and J series in lung cancer cells (Ramer *et al.*, 2013). A further investigation addressing inhibition of glioma stem cell growth demonstrated CBD as a potential 'redox therapeutic' that inhibited glioma stem cell survival, associated with Akt phosphorylation *via* activation of the **p38** MAPK pathway, as well as down-regulation of stem cell marker proteins such as sex-determining region Y-box (Sox)2 and inhibitor of DNA binding (Id)-1, an inhibitor of basic helix–loop–helix transcription factors (Singer *et al.*, 2015). Here, growth inhibition by CBD was mediated *via* production of ROS that induced a regrowth *via* a counter-regulated induction of the antioxidant protein **SLC7A11 (xCT, cystine-glutamate transporter**). The antineoplastic action of CBD was further substantiated by McAllister *et al.* (2011), who demonstrated inhibition of growth and spread of breast cancer cells *via* mitochondrial damage, increased levels of ROS and down-regulation of Id-1. In breast cancer cells, CBD induced intrinsic apoptosis associated with autophagic pathways as indicated by decreased levels of a phosphorylated mechanistic target of rapamycin (**mTOR**), eukaryotic translation initiation factor (eIF) 4E-binding protein 1 and cyclin D1 (Shrivastava *et al.*, 2011).

Recent research concerning induction of cancer cell death has focused on autophagy as an underlying mechanism of cannabinoid-induced antineoplastic action. In this context, THC induces ceramide accumulation, leading to downstream phosphorylation of eIF2α and subsequent endoplasmic reticulum stress associated with autophagy in glioma cells (Salazar *et al.*, 2009). This autophagic effect was, likewise, associated with up-regulation of ATF-4 and TRB3, conferring downstream inhibition of the prosurvival kinase Akt with subsequent inhibition of the mechanistic target of rapamycin complex 1 (mTORC1) and thus induction of autophagic cell death. A contribution of the Akt/mTORC1 axis to a CB_2 receptor-dependent autophagy by THC and the CB_2 receptor agonist JWH015 was further confirmed in experiments using hepatocellular carcinoma *in vitro* and *in vivo* (Vara *et al.*, 2011). In addition to the death-inducing Akt/mTORC1 modulation by cannabinoids, the latter investigation revealed a separate pathway leading to autophagy that encompasses a **calmodulin-activated kinase kinase** subtype, which subsequently phosphorylates **AMP-activated kinase**, thereby conferring autophagy as a response to cannabinoid treatment. Another study was able to demonstrate that cannabinoid-induced TRB3 expression was associated with an up-regulation of PPARγ that appeared essential for an appropriate autophagosome operation, thereby serving as a causal link to cannabinoid-induced autophagic cell death (Vara *et al.*, 2013). Noteworthy, results from these studies suggested autophagy, as a response to cannabinoid treatment, to be involved in the upstream activation of apoptosis rather than acting as apoptosis-alternative pathway leading to cell death (Salazar *et al.*, 2009; Vara *et al.*, 2011). In this context, a recent investigation revealed **TRPV2** as a cannabinoid target, involved in CBD-induced autophagy (Nabissi *et al.*, 2015).

Within the past years, several cannabinoids were found to modulate GPR55, another important key player of cancer progression. GPR55 was found to promote carcinogenesis and was up-regulated in human carcinomas compared with corresponding healthy tissues (Pérez-Gómez *et al.*, 2013). Although the complex network of cannabinoid action on GPR55 signalling requires more data, a recent investigation led authors to pursue the hypothesis of a negative crosstalk between GPR55 and CB_2 receptors and a bidirectional cross-antagonism between both receptors (Moreno *et al.*, 2014).

Besides these antineoplastic effects of exogenously added cannabinoid compounds, a large body of evidence suggests endocannabinoids are similarly able to inhibit cancer cell growth (see Ramer and Hinz, 2016). In agreement with the proposed role of endocannabinoids as cancer-repressive substances, a panel of investigations provided evidence for inhibitors of endocannabinoid turnover to exert comparable effects on cancer cells. In the first publication that revealed a contribution of cannabinoid receptors to the antiproliferative action of AEA, **arachidonoyl-trifluoromethylketone**, an inhibitor of FAAH, enhanced the antiproliferative effects of exogenously

added AEA, while arachidonoyl-trifluoromethylketone alone did not affect the proliferation of breast carcinoma cells (De Petrocellis *et al*., 1998). The concept of inhibition of FAAH as an anticancer strategy was later supported by reports of the FAAH inhibitor, AA-5HT, inhibiting the growth of xenografts generated from thyroid cancer cells (Bifulco *et al*., 2004) and reducing colonic carcinogenesis (Izzo *et al*., 2008). Furthermore, targeting the 2-AG-degrading MAGL has revealed a possible option for suppression of cancer progression. Knockdown of MAGL, using small hairpin RNA and pharmacological inhibition of MAGL with the MAGL inhibitor **JZL184**, suppressed the growth of prostate carcinoma *in vivo via* partial involvement of CB_1 receptors (Nomura *et al*., 2011). Using breast, ovarian and melanoma cancer cells, another study found MAGL inhibition suppressed cancer aggressiveness, without cannabinoid receptor activation, through a decrease of free fatty acids resulting in less cancer-promoting PGs and **lysophosphatidic acid** (Nomura *et al*., 2010). In the latter study, inhibition of cancer growth by MAGL inhibition was reversed by adding back free fatty acids.

Inhibition of cancer growth in a murine xenograft model has further been demonstrated for colorectal carcinoma cells (Ye *et al*., 2011). Here, xenograft growth was inhibited in mice that received colorectal cancer cells transfected with MAGL siRNA or treatment with JZL184. *In vivo* experiments of this investigation revealed knockdown of MAGL, as well as treatment with JZL184 inhibiting cancer cell proliferation and invasion associated with down-regulation of cyclin D1 and **B-cell lymphoma 2** (Bcl-2). Similar results using colorectal carcinoma cells were reported by Ma *et al*. (2016). The latter study found an apoptosis-related decrease of Bcl-2 and increase of **Bcl-2-associated X protein** as a response to treatment with JZL184. However, these studies did not evaluate the contribution of cannabinoid-activated receptors to the observed antiproliferative effects. In another study, lentivirus-mediated MAGL knockdown in HT29 colon cancer cells and MDA-MB-231 breast cancer cells was associated with increased Akt phosphorylation, thereby exhibiting growth inhibition (Sun *et al*., 2013). Again, in this study, phosphorylation of Akt was constitutively suppressed by MAGL, but the contribution of cannabinoid receptors to this phenomenon was not addressed.

Invasion and metastasis

In addition to the numerous investigations demonstrating cannabinoids to elicit antiproliferative and proapoptotic effects on cancer cells, the first report on anti-invasive effects of cannabinoids found CB_1 receptor activation by 2-AG inhibited prostate cancer cell invasion (Nithipatikom *et al*., 2004). Down-regulation of Id-1, a hallmark of cannabinoid-attenuated cancer cell invasion, was observed in CBD-treated breast (McAllister *et al*., 2007) and brain cancer cells (Soroceanu *et al*., 2013). The latter work further confirmed down-regulation of Sox-2, a downstream target of Id-1. Additionally, Id-1 down-regulation, associated with inhibition of cancer cell invasion, was confirmed for the CB_2 receptor agonist O-1663 (Murase *et al*., 2014).

A reduction of cancer cell aggressiveness by Met-F-AEA additionally involved the **Wnt-1** signalling pathway (Laezza *et al*., 2012). Accordingly, the authors showed that treatment of human breast cancer cells with Met-F-AEA decreased nuclear accumulation of **β-catenin** in a CB_1 receptor-dependent manner and was associated with suppression of β-catenin-triggered target oncogenes, such as cyclin D1, c-myc and **MMP-2**. Furthermore, the authors observed suppression of mesenchymal markers, such as vimentin, N-cadherin and **fibronectin**, as well as down-regulation of markers of epithelial-to-mesenchymal transition (EMT), such as Snail1, Slug and Twist. Migration inhibition associated with regulation of the EMT markers, such as down-regulation of vimentin and Snail, as well as up-regulation of E-cadherin, was likewise observed in colorectal cancer cells treated with JZL184 (Ma *et al*., 2016).

Contributions of both CB receptors to the anti-invasive action of THC and, in terms of R(+)-methanandamide, an additional involvement of TRPV1 channels, were demonstrated using cervical and lung cancer cells (Ramer and Hinz, 2008). In the latter report, up-regulation of **tissue inhibitor of matrix metalloproteinases (TIMP)-1** was causally linked to the anti-invasive potential of both cannabinoids and was later confirmed for the anti-invasive impact of CBD on cervical and lung cancer cells (Ramer *et al*., 2010a; Ramer *et al*., 2012). In terms of cannabinoid-induced inhibition of lung cancer cell invasion, TIMP-1 up-regulation occurred *via* upstream induction of the **intercellular adhesion molecule 1 (ICAM-1**) (Ramer *et al*., 2012). In addition to the TIMP-1-dependent anti-invasive properties of phytocannabinoids and R(+)-methanandamide, a recent publication found a causal link between TIMP-1 induction by the FAAH inhibitors, AA-5HT and **URB597**, FAAH siRNA, as well as AEA and OEA and invasion inhibition in lung cancer cells (Winkler *et al*., 2016). Inhibitor experiments pointed towards a role of CB_2 receptors and TRPV1 channels in the anti-invasive effects of FAAH inhibitors and FAAH siRNA. In the same study, both FAAH inhibitors investigated, endocannabinoids (AEA and 2-AG) and endocannabinoid-like substances (PEA and OEA) elicited antimetastatic effects in nude mice.

In other reports, MMP-2 down-regulation was associated with decreased invasion of THC-treated glioma cells (Blázquez *et al*., 2008). Down-regulation of the plasminogen activator inhibitor-1 *via* activation of CB receptors and TRPV1 channels was found using lung cancer cells and is an additional pathway involving CBD's anti-invasive action (Ramer *et al*., 2010b). A recent study further postulated heterodimerization of the chemokine receptor **CXCR4** with the CB_2 receptor, contributing to attenuation of breast cancer cell invasion (Coke *et al*., 2016). Taken together, cannabinoids, as well as inhibitors of endocannabinoid degradation, by virtue of their abilities to modulate regulation of MMPs and to down-regulate Id-1, may present options to specifically suppress the spread of cancer cells.

Angiogenesis

Tumour neovascularization is another important hallmark of cancer progression that was observed to be suppressed by cannabinoid treatment. In this context, data obtained from animal experiments clearly suggest that the antiangiogenic impact of cannabinoids is a general antineoplastic mechanism of this group of substances (see Ramer and Hinz,

2016). Notably, *in vivo* results published by Casanova *et al.* (2003) showed that cannabinoid-induced inhibition of tumour vascularization was associated with down-regulation of the proangiogenic factors: **VEGF**, **placental growth factor** and **angiopoietin-2**. Several other investigations further provided evidence for the inhibition of angiogenic features of endothelial cells following cannabinoid treatment. Antiproliferative and/or antimigratory effects towards HUVEC were reported for WIN 55,212-2 and JWH-133, which were associated with down-regulation of VEGF and MMP-2 (Blazquez *et al.*, 2003). A contribution of MMP-2 down-regulation to antiangiogenic effects was further confirmed for Met-F-AEA (Pisanti *et al.*, 2007) and CBD (Solinas *et al.*, 2012).

In contrast to the consistent data from animal experiments, some *in vitro* studies presented ambiguous cannabinoid effects towards endothelial cells. Accordingly, Kogan *et al.* (2006) reported no antiangiogenic impacts of endocannabinoids on HUVEC proliferation and even proangiogenic effects of low concentrations of THC and CBD. Another study revealed that nanomolar concentrations of AEA stimulated **basic fibroblast growth factor**-induced proliferation of endothelial cells, without affecting proliferation of quiescent endothelial cells (Pisanti *et al.*, 2011). A tendency towards enhanced angiogenic capacities of HUVEC was later confirmed for CBD, THC, R(+)-methanandamide and JWH-133 (Ramer *et al.*, 2014). Thus, some cannabinoids may induce, rather than inhibit, angiogenesis at lower, pharmacologically relevant, concentrations through direct interaction with endothelial cells. This notion, however, contrasts with numerous studies that uniformly reported inhibition of tumour vascularization by cannabinoid compounds *in vivo*. Recent investigations suggest that cannabinoid compounds specifically inhibit angiogenic processes at microenvironmental sites of malignant tissue. In agreement with this assumption, conditioned media from AEA-treated breast cancer cells were found to inhibit endothelial cell proliferation linked to down-regulation of angiogenesis-related factors, such as VEGF, **leptin**, **interferon-γ** and **thrombopoietin** (Picardi *et al.*, 2014). Another study demonstrated that enhanced levels of TIMP-1 in conditioned media of CBD-treated, THC-treated, R(+)-methanandamide-treated and JWH-133-treated lung cancer cells suppressed the angiogenic capacities of endothelial cells (Ramer *et al.*, 2014).

Tumour–immune interactions

Some studies support the hypothesis that cannabinoids may enhance immune responses against the progressive growth and spread of tumours. *In vivo* analyses of melanoma xenograft growth revealed that WIN 55,212-2 produced a more efficient tumour-regressive action in immunocompetent, versus immunodeficient, mice (Blázquez *et al.*, 2006). Another study found CBD, THC and R(+)-methanandamide enhanced the susceptibility of lung cancer cells towards lysis by lymphokine-activated killer cells (Haustein *et al.*, 2014). The underlying mechanism involved a cannabinoid-induced up-regulation of ICAM-1 on the tumour cell surface and a subsequent crosslink with **lymphocyte function-associated antigen-1** on the surface of killer cells. In addition, data from a recent investigation suggested that a reduced infiltration of experimental skin cancer with macrophages and neutrophils in THC-treated animals was associated with tumour regression (Glodde *et al.*, 2015). Thus, cannabinoids may elicit anti-tumour immune responses *via* differential mechanisms, enabling a more effective action of immune cells to combat cancer or by favouring conditions that result in the local reduction of immune cells that cause an inflammatory pro-cancerogenic microenvironment within the cancer tissue. However, one study reported THC increased growth and spreading of mammary carcinoma cells, as a result of inhibition of anti-tumour immune response (McKallip *et al.*, 2005). Therefore, more investigations are necessary to evaluate the effects of cannabinoids on tumour-immune interactions.

Clinical data

Currently, there are no data from large, multicentre, double-blinded, placebo-controlled trials available concerning the systemic anticancer effect of cannabinoids. One clinical pilot study demonstrated that intracranially administered THC was safe in glioblastoma patients (Guzmán *et al.*, 2006). A recently announced exploratory, phase II, randomized, placebo-controlled clinical trial with recurrent glioblastoma multiforme patients provides a signal for the potential efficacy of cannabinoids as add-on anticancer drugs. In this study involving 21 patients, 12 patients were randomized to a combination of THC and CBD in addition to dose-intensive **temozolomide**, whereas the remaining nine patients were randomized to placebo plus standard of care. The proof-of-concept study showed a significantly higher 1 year survival rate in the cannabinoid group, (83 vs. 53% in the placebo cohort). Moreover, the median survival for the cannabinoid group was greater than 550 days compared with 369 days in the group randomized to placebo (GW Pharmaceuticals, 2017 press release).

As a matter of principle, however, the clinical use of cannabinoids may be limited by their psychoactive effects (Wang *et al.*, 2008), as well as by a probable risk for the development of liver fibrosis that has been ascribed to activation of CB_1 receptors (Teixeira-Clerc *et al.*, 2006). Therefore, non-psychoactive substances, such as CBD that exert highly efficient anticancer properties and a considerable safety profile in preclinical experiments, have attracted scientific interest. Notably, a recent study on CBD treatment for Dravet syndrome revealed that a CBD dose of 20 mg per kilogram of body weight per day was associated with somnolence and elevation of liver enzyme levels, when compared with the control group (Devinsky *et al.*, 2017). As the placebo and the CBD treatment groups received several additional antiepileptic drugs, adverse interactions with CBD, rather than a toxicity of CBD *per se*, were discussed by the authors as a possible cause of the observed adverse effects. Furthermore, CB_2 receptor agonists as non-psychoactive cannabinoids have gained scientific interest. In this context, it is noteworthy that, in contrast to the profibrotic CB_1 receptor activation (Teixeira-Clerc *et al.*, 2006), CB_2 receptor agonists have been found to exert antifibrotic effects on the liver (Julien *et al.*, 2005).

Combinational effects with chemotherapeutic agents

Taking into account that future clinical studies addressing systemic anticancer effects of cannabinoids will most likely not be conducted as monotherapy, but in combination with established chemotherapeutics, recent research concerning probable interactions between cannabinoids and currently used cytostatic drugs suggests cannabinoids as notable additives to a number of chemotherapeutic effects. Figure 2 provides an overview on this subject. As recently summarized, CBD and THC enhance the cytotoxic effects of several chemotherapeutic agents, including vinca alkaloids, **cytarabine**, **doxorubicin**, **mitoxantrone**, **carmustine**, temozolomide, **bortezomib**, **carfilzomib** and **cisplatin** (Ramer and Hinz, 2017). Thus, THC and CBD have both been shown to enhance the cytostatic effect of **vinblastine** in resistant leukaemia cells *via* down-regulation of **P-glycoprotein** (Holland *et al.*, 2006) and of mitoxantrone in embryonic fibroblasts through inhibition of **ABCG2** (Holland *et al.*, 2007). An enhancement of the cytostatic properties of cytarabine, doxorubicin and **vincristine** has, likewise, been demonstrated for THC in leukaemia cells (Liu *et al.*, 2008). The sensitization of cells appeared to be dependent on decreased p42/44 MAPK activity in response to sublethal concentrations of THC. In addition, a decreased chemoresistance of myeloma cells towards the proteasome inhibitor bortezomib was observed when combined with CBD (Morelli *et al.*, 2014). The toxic effect of CBD alone, or in combination with bortezomib, was found to exert higher efficacy in multiple myeloma cells that express TRPV2 channels, when compared with TRPV2-deficient cells. A crucial role for TRPV2 channels in the reduction of chemoresistance has further been confirmed for carmustine, doxorubicin and temozolomide in CBD-challenged glioma cells (Nabissi *et al.*, 2013), as well as for doxorubicin in triple negative breast cancer cells treated with CBD (Elbaz *et al.*, 2016). THC and CBD, or a combination of both, were further demonstrated to enhance the toxic impact of temozolomide on glioma cells *in vitro* and *in vivo via* a mechanism that involves autophagy (Torres *et al.*, 2011). Notably, data from this investigation were one basis for the aforementioned placebo-controlled, phase II study demonstrating that a combination of THC and CBD produced a prolonged survival when combined with temozolomide in glioblastoma patients. Synergistic actions were further reported for the effect of a CBD/THC combination added to multiple myeloma cells in the presence of carfilzomib. The cannabinoids here were shown to overcome resistance to carfilzomib by reducing the proteasome β5i subunit at both the transcriptional and translational levels (Nabissi *et al.*, 2016). The susceptibility of glioblastoma cells to the cytotoxic action of cisplatin was further found to be enhanced by CBD (Deng *et al.*, 2017).

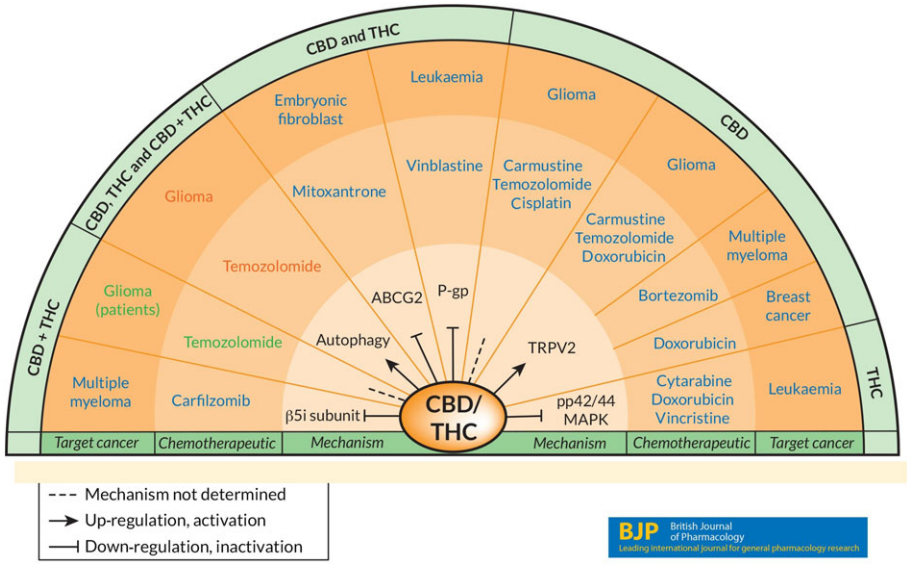

Figure 2

Enhancement of the effects of chemotherapeutic agents on cancer cells or tissue by CBD and/or THC. Arrows in the inner layer specify cannabinoid-induced up-regulation/activation or down-regulation/inactivation of the indicated parameters or mechanisms involved in the anti-tumour effects of the combination with the indicated anticancer drug in the second layer, respectively. The third layer specifies the cancer type, and the outer layer shows the respective treatment. Dashed lines indicate investigations where no mechanism was assessed. Drugs and cancer types in blue letters indicate cell culture studies that addressed cannabinoid-induced enhanced sensitivity of cancer cells towards the cytotoxic action of the corresponding chemotherapeutic agents. Red letters indicate a study that demonstrated an enhancement of the cancer-regressive effect of temozolomide by THC, CBD and a THC/CBD combination and further included *in vivo* experiments using a murine xenograft model. Details of these preclinical evaluations and references are indicated under the section Combinational effects with chemotherapeutic agents. Green letters specify temozolomide effects boosted by combination with CBD/THC assessed in a phase II, randomized, placebo-controlled clinical trial with recurrent glioblastoma multiforme patients (GW Pharmaceuticals, 2017 press release). Details concerning this clinical study are described in the section on Clinical data.

Notably, investigations concerning the beneficial interactions between cannabinoids and chemotherapeutics were not confined to synergistic anticancer actions and also addressed probable suppression of adverse chemotherapeutic effects by cannabinoids. Thus, benefits for some cancer patients with chemotherapy-induced peripheral neuropathy (Gingerich *et al.*, 2009) have been reported under treatment with cannabinoids. Moreover, cannabinoids such as CBD (Pan *et al.*, 2009) and the peripherally restricted cannabinoid CB_2 receptor agonist LEI-101 (Mukhopadhyay *et al.*, 2016) attenuated cisplatin-induced nephrotoxicity in mice.

Outlook

Taken together, pharmacological use of cannabinoid receptors and other components of the endocannabinoid system, in the broader sense, represents an attractive option for anticancer therapy, at least at the preclinical level. As a matter of fact, the endocannabinoid system offers a broad spectrum of targets that influence cancer cell fate. Particular scientific interest has been attracted by non-psychoactive cannabinoids such as CBD, which has been demonstrated to exert a broad array of anticarcinogenic properties, such as antiproliferative action towards cancer cells (Ligresti *et al.*, 2006), anti-invasive and antimetastatic effects (Ramer *et al.*, 2010a, 2012) as well as induction of apoptosis (Massi *et al.*, 2004) and autophagy (Shrivastava *et al.*, 2011; Nabissi *et al.*, 2015). The spectrum of the cancer-regressive action of CBD is further complemented by its property to enhance the susceptibility of resistant cancer cells towards chemotherapeutic agents (Holland *et al.*, 2006) and to inhibit angiogenesis (Solinas *et al.*, 2012). As a further treatment option for systemic cancer therapy, modulation of endocannabinoid-degrading enzymes has gained considerable interest. There is a consistent line of evidence suggesting that inhibitors of FAAH exert cancer-suppressive actions, while sparing psychoactive effects. For instance, FAAH inhibitors have been found to lack hypomotility (Schlosburg *et al.*, 2009). Another investigation found dual inhibition of FAAH and MAGL mimicked the effects of direct CB_1 receptor agonists, such as THC (Long *et al.*, 2009). The latter study further reported that the MAGL inhibitor JZL184 induced THC-like drug discrimination responses, which may reflect a contribution of the MAGL/2-AG pathway to mimic THC-like psychoactive effects.

When a high percentage of fatal cancer progressions results from intravasation and extravasation of cancer cells, it highlights the importance of new drugs that efficiently block metastasis of malignant neoplastic cells. In view of the large number of animal studies that demonstrate cannabinoid compounds to exert antimetastatic effects, these substances may serve as feasible add-on options for the currently used cytostatics. This view is further substantiated by a number of preclinical investigations that provide evidence for the beneficial synergistic action of cannabinoids combined with chemotherapeutic drugs. However, potential adverse drug interactions must be carefully evaluated in future studies. Additionally, cannabinoids may provide a pharmacotherapeutic option as low molecular weight inhibitors of tumour neovascularization in addition to the currently used TK inhibitors **sunitinib**, **pazopanib**, **sorafenib**, **axitinib** and **bevacizumab** that improve overall survival of patients with different cancer types, such as renal cell carcinoma.

Future clinical studies that permit reliable conclusions from successful bench-to-bedside translation are urgently needed, particularly, since the passage of new laws allowing patient access to cannabinoids and the growing market of cannabinoid-based nutraceuticals. Scientists must quickly gather facts concerning the risks and benefits of cannabinoid compounds for cancer patients, or else the non-scientific media will create its own 'facts'.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017a,b,c,d).

Conflict of interest

The authors declare no conflicts of interest.

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