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The use of cannabinoids as anticancer agents

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ABSTRACT

It is well-established that cannabinoids exert palliative effects on some cancer-associated symptoms. In addition evidences obtained during the last fifteen years support that these compounds can reduce tumor growth in animal models of cancer. Cannabinoids have been shown to activate an ER-stress related pathway that leads to the stimulation of autophagy-mediated cancer cell death. In addition, cannabinoids inhibit tumor angiogenesis and decrease cancer cell migration. The mechanisms of resistance to cannabinoid anticancer action as well as the possible strategies to develop cannabinoid-based combinational therapies to fight cancer have also started to be explored. In this review we will summarize these observations (that have already helped to set the bases for the development of the first clinical studies to investigate the potential clinical benefit of using cannabinoids in anticancer therapies) and will discuss the possible future avenues of research in this area.

Contents

1. Introduction

Δ^9-tetrahydrocannabinol (THC), the main active component of *Cannabis sativa* exerts its effects by mimicking endogenous substances – the endocannabinoids anandamide (Devane et al., 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam et al., 1995; Sugiura et al., 1995) – that bind specific cannabinoid receptors located in the plasma membrane (Pertwee et al., 2010). Two major cannabinoid-specific receptors – CB_1 and CB_2 – have been identified (Matsuda et al., 1990; Munro et al., 1993). The transient receptor potential cation channel subfamily V member 1 (TRPV1), the orphan G protein-coupled receptor GPR55 and peroxisome proliferator-activated receptors (PPARs) have been proposed to act as endocannabinoid receptors, although their

Abbreviations: 2-AG, 2-arachidonoylglycerol; ALK, anaplastic lymphoma kinase; ATF-4, activating transcription factor 4; CB_1, cannabinoid CB_1 receptor; CB_2, cannabinoid CB_2 receptor; CBD, cannabidiol; CHOP, C/EBP homologous protein; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; MDK, midkine; mTORC1, mammalian target of rapamycin complex 1; THC, Δ^9-tetrahydrocannabinol; TRIB3, tribbles-homologue 3; TRPV1, transient receptor potential cation channel subfamily V member 1 (TRPV1); VEGF, vascular endothelial growth factor.

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precise contribution in the context of the endocannabinoid signaling is still a matter of debate (Pertwee et al., 2010). Most of the cannabinoids effects in the central nervous system rely on CB_1 receptor activation (Pertwee et al., 2010), Nevertheless expression of CB_1 receptor is not restricted to the central nervous system and this receptor is widely expressed in many different locations in the organism (Pertwee et al., 2010) The CB_2 receptor was initially described to be present in the immune system (Pertwee et al., 2010), although different studies have shown that it is also present in cells from other origins including astrocytes and certain populations of neurons (Atwood and Mackie, 2010; Fernandez-Ruiz et al., 2007). Of note, expression of CB_1 and CB_2 receptors occurs in many types of cancer cells, an event that not necessarily correlates with the expression of these receptors in non-transformed cells from the tissue from which cancer cells originated (Fernandez-Ruiz et al., 2007; Guzman et al., 2006; Sarfaraz et al., 2008).

The endocannabinoid system – constituted by the endocannabinoids, their receptors and the proteins involved in the synthesis, transport and degradation of endocannabinoids – exerts numerous regulatory functions in the organism (Katona and Freund, 2008); (Pacher et al., 2006; Pertwee, 2009). Accordingly, the pharmacological manipulation of the endocannabinoid system is being investigated for the treatment of many different diseases. In a cancer context, cannabinoids have been shown to alleviate nausea and vomit induced by chemotherapy (Guzman, 2003; Pertwee, 2009) and several cannabinoid-based medicines [Marinol (THC) and Cesamet (nabilone, a synthetic analogue of THC)] are approved for this purpose. Cannabinoids also inhibit pain, and Sativex (a standardized cannabis extract) has been approved in Canada for the treatment of cancer-associated pain. Other potential palliative effects of cannabinoids in oncology include appetite stimulation and attenuation of wasting (Pertwee et al., 2010).

In addition to these palliative actions of cannabinoids in cancer patients, THC and other cannabinoids exhibit antitumor effects in animal models of cancer (Guzman, 2003; Sarfaraz et al., 2008); (Pisanti et al., 2013; Velasco et al., 2012).

2. Endocannabinoid system: role in tumor generation and progression

A relatively large body of data has accumulated during the last decade about the role of endocannabinoid system in tumor generation and progression (see Table 1 for a brief summary of some of these observations). In many cases, these reports show that levels of endocannabinoids and their receptors are increased in cancer, a situation that frequently correlates with tumor aggressiveness (Malfitano et al., 2011). Accordingly, anandamide and 2-AG have been shown to be over-expressed in several types of tumors including glioblastoma multiforme (GBM), meningioma, pituitary adenoma, prostate and colon carcinoma and endometrial sarcoma (Pisanti et al., 2013). In addition, circulating endocannabinoid levels have been associated with increased disease progression in a mouse model of metastatic melanoma and in human samples of this pathology (Sailler et al., 2014). A similar situation has been proposed for cannabinoid receptors and endocannabinoid degrading enzymes. Thus, CB1 receptor was found to be upregulated in Hodgkin lymphoma cells (Benz et al., 2013) and in chemically induced cellular hepatocarcinoma (Mukhopadhyay et al., 2015). CB1 receptor levels are also increased and correlate with disease severity in human epithelial ovarian tumors (Messalli et al., 2014) and have been proposed to be a factor of bad prognosis following surgery in stage IV colorectal cancer (Jung et al., 2013).

Regarding CB2 receptor, a correlation between its expression, histologic grade and prognosis has been demonstrated in breast cancer (Caffarel et al., 2006) and glioma (Sanchez et al., 2001). In this latter tumor type a combined up-regulation of CB1 and CB2 receptors has been proposed to occur together with a decrease on the levels of the enzymes involved in endocannabinoid degradation compared to healthy controls (Wu et al., 2012). Similarly, expression of CB1 and CB2 is enhanced in mantle cell lymphoma, while FAAH expression is reduced compared to non-malignant B-cells (Ek et al., 2002; Islam et al., 2003; Wasik et al., 2014).

Recently, a role for the non-canonical cannabinoid receptor GPR55 in cancer development has been described. Higher histological grades of human glioblastomas, breast, pancreatic and skin cancers have been reported in association with increased GPR55 expression. Moreover, silencing of GPR55 reduced the proliferation of tumor cells in a xenograft mouse model of glioblastoma (Andradas et al., 2011; Perez-Gomez et al., 2013).

Altogether, these data suggest that the endocannabinoid system may play a pro-tumorigenic role and in agreement with this hypothesis genetic ablation of CB1 and CB2 receptors decreases UV light induced skin carcinogenesis (Zheng et al., 2008) and CB2 receptor overexpression enhances the predisposition to leukemia after leukemia virus infection (Joosten et al., 2002). Moreover, genetic ablation of CB1 receptor

Table 1
Changes in the expression of cannabinoid (CB) receptors or endocannabinoids (ECB)-degrading enzymes in human cancer.

Tumor type	CB receptors or ECB degrading enzymes	References
Hodgkin lymphoma	CB1 levels increased	(Benz et al., 2013)
Non-Hodgkin lymphoma	CB1 levels increased	(Gustafsson et al., 2008)
Chemically-induced cellular hepatocarcinoma	CB1 levels increased	(Mukhopadhyay et al., 2015)
Hepatocellular carcinoma	CB1 and CB2 expression correlates with improved prognosis of patients with hepatocellular carcinoma	{Xu, 2006 #378}
Human epithelial ovarian tumors	CB1 levels increased. Correlation with disease severity	(Messalli et al., 2014)
Stage IV colorectal cancer	CB1 levels are a factor of bad prognosis following surgery	(Jung et al., 2013)
Colon cancer	CB1 levels decreased, CB1 genetic ablation increases the growth of colon carcinomas	(Wang et al., 2008)
Pancreatic cancer	CB1 and CB2 levels increased and MAGL and FAAH levels decreased associated with bad prognosis	(Michalski et al., 2008)
Prostate cancer	CB1 levels increased associated with severity of disease and poor prognosis	(Chung et al., 2009)
Prostate cancer	FAAH tumor levels (but not CB1) directly correlate with severity of the diseases	(Thors et al., 2010)
Breast cancer	CB2 levels increased. Correlation with disease severity	{Caffarel, 2010 #15;Caffarel, 2006 #16;Perez-Gomez et al., 2015 #349}
Glioma	CB2 levels increased with degree in gliomas	(Sanchez et al., 2001)
Mantle cell lymphoma	CB1 and CB2 levels increased and FAAH levels decreased	(Ek et al., 2002; Islam et al., 2003; Wasik et al., 2011)
UV light induced skin carcinogenesis	CB1 and CB2 genetic ablation decrease UV light induced skin carcinogenesis	(Zheng et al., 2008)
Leukemia	CB2 overexpression enhances the predisposition to leukemia after leukemia virus infection.	(Joosten et al., 2002)
Glioma, breast cancer, skin cancer	GPR55 increased levels associated with higher histological tumor grade	(Andradas et al., 2011; Perez-Gomez et al., 2013)

suppresses the growth of hepatocellular carcinoma (Mukhopadhyay et al., 2015).

Nevertheless, different observations also support that the endocannabinoid system plays a tumor suppressor role in different cancer types. Thus, genetic inactivation of CB1 receptor increases intestinal tumor growth in a colon carcinoma genetic mouse model (Wang et al., 2008). In line with this idea, monoacylglycerol lipase (MAGL; the 2-AG degrading enzyme), has been shown to be highly expressed in several types of tumors, which is associated with increased migration, invasion, survival, and tumor growth (Nomura et al., 2010). In addition, FAAH tumor levels directly correlate with the severity and outcome of prostate adenocarcinoma (Thors et al., 2010). These data are in line with accumulative evidences (described in the following section), that demonstrate that cannabinoids (endogenous, phytocannabinoids or synthetic) act as efficient anti-tumoral agents in a wide range of cancer cells.

Further studies, including those analyzing the activation of the precise signaling mechanisms involved in the regulation of cannabinoid-induced cell death or cell proliferation upon genetic or pharmacological manipulation of the endocannabinoid system, are therefore needed to clarify which are the determinants for this system to act as oncogenic or tumor suppressor.

3. Cannabinoid anticancer activity

Despite the above discussed conflicting data relative to the role of endocannabinoid system in tumor generation and progression, during the last fifteen years many different reports have shown that cannabinoid receptor agonists (derived from the plant, like THC, endogenous like 2-AG and anandamide or synthetic — with similar or different affinity for CB_1 and CB_2 receptors like WIN 55,2121-2 or JWH-133) exert antitumor effects in experimental models of cancer [reviewed in Velasco et al. (2012)] supporting that pharmacological stimulation of CB receptors is antitumorigenic. Nonetheless, a tumor-promoting effect of cannabinoids has been proposed in few reports (Cudaback et al., 2010; Hart et al., 2004; McKallip et al., 2005; Zhu et al., 2000).

Cannabinoid treatment promotes cancer cell death, impair tumor angiogenesis and block invasion and metastasis (Velasco et al., 2012). The molecular mechanisms that have been proposed to be involved in cannabinoid anticancer actions have been thoroughly reviewed elsewhere (Caffarel et al., 2012; Pisanti et al., 2013; Velasco et al., 2012) and therefore will only be shortly discussed here.

3.1. Cannabinoids induce cancer cell death

The mechanism of cannabinoid anticancer action relies, at least largely, on the ability of these agents to stimulate autophagy-mediated apoptotic cancer cell death (Velasco et al., 2012). Thus, THC binds cannabinoid receptors, which leads to the stimulation of sphingolipid synthesis de novo and the subsequent activation of an ER stress-related signaling route that involves the up-regulation of the transcriptional co-activator nuclear protein 1 (Nupr1, also named p8) and its effector the pseudokinase tribbles homolog 3 (TRIB3) (Armstrong et al., 2015; Blazquez et al., 2004; Carracedo et al., 2006a,b; Galve-Roperh et al., 2000; Gomez del Pulgar et al., 2002; Velasco et al., 2012). The stimulation of this pathway promotes in turn autophagy via TRIB3-mediated inhibition of the AKT/mTORC1 axis (Salazar et al., 2009; Salazar et al., 2013). Autophagy is considered primarily a cytoprotective mechanism, although its activation can also lead to cell death (Eisenberg-Lerner et al., 2009; Galluzzi et al., 2015; Mizushima et al., 2008). A series of experiments demonstrated that autophagy is upstream of apoptosis in the mechanism of cannabinoid-induced cell death (Armstrong et al., 2015; Salazar et al., 2009; Vara et al., 2011).

The direct participation of the autophagy pathway in the antitumor action of cannabinoids has been clearly demonstrated in different types of cancer cells [namely, glioma, melanoma, pancreatic and hepatic cancer cells (Armstrong et al., 2015; Carracedo et al., 2006a,b; Salazar et al., 2009; Vara et al., 2011)]. These observations support that this signaling route could be a general mechanism by which activation of CB receptors

Box 1

Mechanism of cannabinoid receptor-mediated cancer cell death: some important unanswered questions.

Research performed in the last decade has permitted a better understanding of the intracellular signaling mechanisms underlying cannabinoid anticancer action. However, a number of important observations remain to be clarified. For example:

- Unlike the cell death-promoting action of cannabinoids on cancer cells, the viability of normal (non-transformed) cells is unaffected or – under certain conditions – even enhanced by cannabinoid challenge (Carracedo et al., 2006b; Galve-Roperh et al., 2000, 2008; Gomez del Pulgar et al., 2002; Salazar et al., 2009). For example, THC treatment of astrocytes (a cell type that expresses functional CB_1 receptors) does not trigger the activation of ER stress, the up-regulation of the p8 pathway, the inhibition of the AKT–mTORC1 axis or the stimulation of autophagy and apoptosis, even when concentrations of THC higher than those that promote glioma cell death are used (Carracedo et al., 2006b; Salazar et al., 2009). Similar results were obtained with primary embryonic fibroblasts (Carracedo et al., 2006b; Salazar et al., 2009) and other types of non-transformed cells expressing functional cannabinoid receptors when compared with their transformed counterparts (Blazquez et al., 2006; Caffarel et al., 2006; Casanova et al., 2003; Chan et al., 1996). Thus, stimulation of cannabinoid receptors seems to be coupled to the activation of different signaling mechanisms in transformed and non-transformed cells. The precise molecular reasons responsible for this differences remain as an one of the unanswered questions within the cannabinoid field that still require much further research in order to be clarified.
- Another puzzling observation is that pharmacological inhibition of either CB_1 or CB_2 receptors prevents THC-induced cell death at least in certain cancer cells (for example glioma cells) (Galve-Roperh et al., 2000; Lorente et al., 2011), whereas in, hepatic (Vara et al., 2011), pancreatic (Carracedo et al., 2006a) or breast (Caffarel et al., 2006) carcinoma cells, antagonists of CB_2 but not of CB_1 receptors inhibit cannabinoid anticancer actions.
- Certain cannabinoid receptor agonists trigger cancer cell death more efficiently than others exhibiting even higher affinity for CB receptors. Thus, THC promotes cancer cell death (an effect that can be blocked using of CB receptors antagonists) at lower concentrations than WIN-55,212-2 [a cannabinoid receptor agonist which exhibits in binding assays higher affinity than THC for CB_1 and CB_2 receptors (Pertwee et al., 2010)].

Recent observations suggest that CB_2 and GPR55 receptors can form heteromers — and that these structures can modify the antitumoral activity of cannabinoids (Moreno et al., 2014). Whether some of the intriguing effects described above can be explained by the ability of cannabinoid receptors to oligomerize with other G protein-coupled receptors, locate in precise domains in the plasma membrane (or in organelles) or couple to specific G proteins or other signaling molecules are interesting possibilities that require much further research.

promotes cancer cell death. In any case, additional mechanisms (some of them cell type specific) may cooperate with this pathway to trigger cancer cell death (Vara et al., 2011; Caffarel et al., 2006, 2012; Guzman, 2003; Sarfaraz et al., 2008; Vara et al., 2013). (see also Box 2).

Cannabidiol [CBD; a plant-derived cannabinoid with low affinity for cannabinoid receptors; (Pertwee, 2009)], and other marijuana-derived cannabinoids (Ligresti et al., 2006) have also been shown to trigger apoptosis in cancer cells. CBD produces these anticancer actions – at least in part – via enhanced production of reactive oxygen species (Massi et al., 2008; Shrivastava et al., 2011). It has also been proposed that CBD may activate TRPV2 receptors to promote cancer cell death (Nabissi et al., 2012).

3.2. Cannabinoids inhibit angiogenesis, invasion and metastasis

In addition to the above-described cancer cell death promoting effect of cannabinoids, treatment with these compounds has been shown to normalize tumor vasculature. These effects seem to rely on the ability of cannabinoids to inhibit the stimulation of the vascular endothelial growth factor (VEGF) pathway. Thus, various components of the VEGF-activated pathway, such as the active forms of its best-established receptors (VEGFR1 and VEGFR2), have been shown to be down-regulated in response to treatment with cannabinoids in different cancer types (Casanova et al., 2003; Blazquez et al., 2003, 2004; Portella et al., 2003). Likewise, cannabinoid receptor activation inhibits migration and proliferation, and induces apoptosis in vascular endothelial cells (Blazquez et al., 2003; Pisanti et al., 2007) which might also contribute to the antiangiogenic effect of cannabinoids.

In addition, cannabinoids have been shown to reduce the formation of distant tumor masses in animal models of spontaneous and induced metastasis. Moreover, these compounds inhibit migration, adhesion and invasiveness of different types of cancer cells (Blazquez et al., 2008; Grimaldi et al., 2006; Preet et al., 2008; Qamri et al., 2009; Ramer and Hinz, 2008). This anti-metastatic activity of cannabinoids relies, at least in part, on the regulation of extracellular proteases and their inhibitors (Blazquez et al., 2008; Ramer and Hinz, 2008). Several observations support that the ER stress-related signaling pathway involved in the stimulation of autophagy-mediated cancer cell death may also play a role in the control of these actions of cannabinoids (Blazquez et al., 2004, 2008).

Of note, CBD exerts a significant anticancer effect – and specifically the inhibition of invasiveness and mestastasis – in different animal models of cancer acting independently of cannabinoid receptors. This effect of CBD relies – at least partially – on the downregulation of ID-1 (transcription factor inhibitor of DNA binding-1) (McAllister et al., 2011; Murase et al., 2014; Soroceanu et al., 2012).

4. Mechanisms of resistance to cannabinoid anticancer action

Today it is well established that the molecular characteristics of each individual tumor and patient determine the responsiveness to anticancer therapies. Although much further research is still required to clarify this issue in the case of cannabinoids, work performed in our laboratory supports that – at least in gliomas – the differences in the expression of a particular set of genes rather than in the levels of CB receptors determine the sensitivity to THC-induced cell death, (Lorente et al., 2011). We found that increased expression of midkine [MDK; (Kadomatsu, 2005; Mirkin et al., 2005), one of the genes that is strongly up-regulated in cannabinoid-resistant glioma cells] is associated with a lower overall survival of glioblastoma patients (Lorente et al., 2011). MDK promotes resistance to THC-induced cell death via stimulation of one of its target receptors, the anaplastic lymphoma tyrosine kinase receptor [ALK (Palmer et al., 2009)] which abrogates the induction of autophagy-mediated glioma cell death by THC. Supporting the potential therapeutic relevance of these findings, pharmacological inhibition of ALK or MDK knock-down abolishes the resistance to cannabinoid treatment of tumor xenografts derived from THC-resistant glioma cells (Lorente et al., 2011). Altogether, these observations support that stimulation of the MDK–ALK axis promotes resistance to cannabinoid anticancer action in glioblastoma and paves the way for the development of anticancer therapies based on the combined administration of THC and inhibitors of the MDK–ALK axis (Fig. 1). In line with this idea, ALK inhibitors – which have started to be assayed in clinical trials for the management of non-small-cell lung cancer and other types of

Box 2

Different pharmacological approaches to target cancer cells with cannabinoids.

Cannabinoid agonists or enhancers of endocannabinoid tone?

Administration of endocannabinoids or inhibitors of endocannabinoid-degrading enzymes has been shown to reduce the growth of different types of tumor xenografts (Bifulco et al., 2001; Ligresti et al., 2003) and, therefore, could be a reasonable strategy for targeting cannabinoid receptors for anticancer purposes. However, as discussed in section 2, the role of the endocannabinoid system, including the endocannabinoid-degrading enzymes, in the control of tumor generation and progression is not well understood. Since enhancing endocannabinoid tone only has mild anti-tumor effects in mice and since no inhibitor of endocannabinoid degradation has been approved as yet for use in humans, clinical studies aimed at analyzing the efficacy of cannabinoids as anti-tumor agents should be based on the use of plant-derived or synthetic agonists of cannabinoid receptors rather than on endocannabinoids or inhibitors of endocannabinoid degradation.

Cannabis extracts or pure cannabinoids?

The long-known therapeutic properties of *Cannabis sativa* – including amelioration of symptoms associated with cancer and its chemotherapy – have led to the authorization of the medical use of this plant and its extracts in several countries. As mentioned in the text, some of the other cannabinoids present in marijuana may contribute to the attenuation of THC psychoactive-side effects (Pertwee, 2009) However, pure drugs are more prone to standardization than complex molecular cocktails. Thus, it would be ideal that studies aimed at investigating the anticancer actions of cannabinoids in patients were performed comparatively with both pure substances and cannabis extracts containing controlled amounts of THC, CBD and other cannabinoids.

Which routes of cannabinoid administration?

Smoking is the most frequent route of administration of self-medicated and recreational marijuana. Thus, THC and other cannabinoids derived from the plant are rapidly absorbed by inhalation. However, smoking is an unattractive clinical option. In the first clinical trial in which a cannabinoid was assayed as an anti-caner agent, THC was administered locally (intracranial delivery to GBM patients) (Guzman et al., 2006). Nevertheless, this route of administration has many obvious limitations. Currently-available cannabis-based medicines are administered as capsules or using an oro-mucosal spray (Pertwee, 2009). Preclinical animal models have yielded data indicating that systemic (oral or intraperitoneal) administration of cannabinoids effectively reduces tumor growth (author's unpublished observations). Thus, it seems reasonable that future clinical studies directed at determining the efficacy of cannabinoids as anti-cancer agents use oral or oro-mucosal routes of administration.

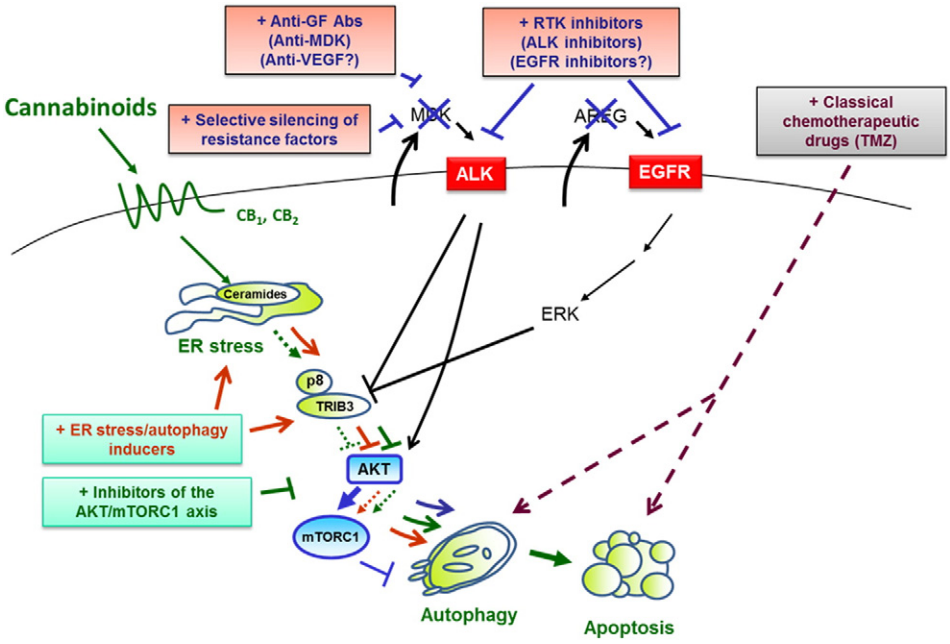

Fig. 1. Possible strategies aimed at optimizing cannabinoid-based therapies against gliomas. Resistance of glioma cells to cannabinoid-induced cell death relies, at least in part, on the enhanced expression of the growth factor midkine (MDK) and the subsequent activation of the anaplastic lymphoma receptor tyrosine kinase (ALK). Enhanced expression of amphiregulin (AREG, a heparin-bound ligand of the EGFR) can promote resistance to THC antitumor action via ERK stimulation. Combination of THC with pharmacological inhibitors of ALK (or genetic inhibition of MDK) enhances cannabinoid action in resistant tumors. Combinations of cannabinoids with classical chemotherapeutic drugs such as the alkylating agent temozolomide [TMZ; the benchmark agent for the management of glioblastoma (Lonardi et al., 2005; Stupp et al., 2005)] produce a strong anticancer action in animal models. Other strategies to enhance cannabinoid anticancer action could be combining cannabinoids with endoplasmic reticulum (ER) stress and/or autophagy inducers or with inhibitors of the AKT-mechanistic target of rapamycin C1 (mTORC1) axis. Abs: antibodies; EGFR: epidermal growth factor receptor; ERK: extracellular signal-regulated kinase; GF: growth factors; RTK: receptor tyrosine kinase; TRIB3: tribbles 3; VEGF: vascular endothelial growth factor.

tumors (de Bono and Ashworth, 2010; Grande et al., 2011) – have been proposed to be of potential utility in glioblastoma multiforme (GBM) (Wallace et al., 2013). Following this line of reasoning, the ALK and the MET proto-oncogene, receptor tyrosine kinase (MET) inhibitor *Crizotinib* is currently being evaluated in combination with radiotherapy and temozolomide [TMZ; the benchmark agent for the management of glioblastoma (Stupp et al., 2005)] in a Phase 1b clinical study in adult patients with newly diagnosed glioblastoma (NCT02270034) which may facilitate the development of future studies combining this inhibitor with cannabinoids. A second generation of ALK inhibitors with a lower risk of developing drug resistance in patients, such as *Ceritinib or Alectinib*, is being already evaluated in clinical studies (Pall, 2015). Alternative approaches to inhibit MDK–ALK axis could also include the use of humanized antibodies against MDK or its receptor ALK.

It is worth noting that other growth factors [such as the heparin-bound epidermal growth factor receptor (EGFR) ligand amphiregulin] have been implicated in the resistance to cannabinoid antitumor action (Lorente et al., 2009; Hart et al., 2004). Thus pharmacological blockade of EGFR, (Lorente et al., 2009) enhances the cell death-promoting action of THC in cultures of glioma cells. These observations suggest that targeting EGFR pathway may also be a therapeutic strategy to enhance cannabinoid anticancer activity. Whether these or other mechanisms may play a relevant role in promoting resistance to cannabinoid anticancer action in other tumor types remain to be investigated.

5. Towards the use of cannabinoid-based combinational therapies

Current strategies to fight cancer are based on the use of combinational anticancer therapies as this approach permits the simultaneous targeting of tumor growth, at different levels. In agreement with this line of reasoning, the combined administration of cannabinoids with other anticancer agents has been shown to act synergistically to inhibit tumor growth. Accordingly, treatment with THC and TMZ exerts a strong anti-cancer action in xenografts generated with glioma cells. Importantly this effect also takes place in TMZ-resistant tumors (Torres et al., 2011). Likewise, mice treated with TMZ and THC did not show signs of toxicity (Torres et al., 2011). Most glioblastoma patients are treated with TMZ, and therefore these findings support that the combined administration of TMZ and cannabinoids could be therapeutically exploited for the management of glioblastoma (Fig. 1).

Likewise, another study performed with pancreatic cancer cells showed that gemcitabine (the benchmark agent for the treatment of pancreatic cancer) acted synergistically with different cannabinoid agonists to reduce cell viability (Donadelli et al., 2011). Other studies showed that anandamide and HU-210 increase the antineoplastic activity of paclitaxel (Miyato et al., 2009) and 5-fluorouracil (Gustafsson et al., 2009).

Another approach has been to assay the anticancer activity of the combination of THC and CBD. Thus, the administration of these two agents enhances the anticancer activity of THC and decreases the doses of THC required to produce tumor growth-inhibition (Marcu et al., 2010; Torres et al., 2011). Moreover, the combined administration of THC, CBD and TMZ produces a very strong decrease in the growth of xenografts generated with glioma cells even when low doses of THC are employed (Torres et al., 2011). Furthermore the administration of THC and CBD also enhanced the anticancer effects of radiation in an orthotopic murine glioma model (Scott et al., 2014). Since, CBD alleviates some of the undesired side effects of THC (for example discoordination, convulsions, and psychotic events), its administration in combination with THC may help to improve the tolerability to medicines containing this agent or other cannabinoid receptor agonists (Pertwee, 2009). Following this line of reasoning it is worth noting that *C. sativa* produces ~108 different cannabinoids and, apart from CBD, some of them may help to reduce the undesired side-effects of THC or have other therapeutic activities (Pertwee, 2009). Therefore, in addition to the use of pure substances (such as THC and CBD) for the development of clinical studies to investigate the efficacy of cannabinoids as anticancer agents, one possible additional approach could be using

cannabis extracts with precisely-defined amounts of THC, CBD and other cannabinoids.

6. Towards the development of clinical studies to test the efficacy of cannabinoids as anticancer agents

Despite the remarkable amount of preclinical research on the potential therapeutic applications of cannabinoids the use of cannabis-based medicines in the clinical practice is restricted to palliative uses in a few diseases. Nevertheless, preclinical data accumulated during the last decade has stimulated the interest in developing additional clinical studies aimed at investigating the potential therapeutic value of these compounds in different diseases and specifically their potential as anticancer agents. The first of this studies was a pilot Phase I clinical trial in which 9 patients with actively-growing recurrent glioblastoma that had previously failed standard therapy underwent intracranial THC administration (Guzman et al., 2006). Cannabinoid delivery under these conditions was safe. Likewise, significant undesired effects were not observed in the patients of the study. In addition, analysis of the results obtained in this study suggested that some patients responded – at least partially – to THC treatment (Guzman et al., 2006). Importantly, analyses of samples obtained from 2 patients in this study before and after THC treatment indicated that administration of this cannabinoid correlated with the activation of the mechanisms that had been previously shown to be involved in the anticancer activity of THC in animal models of cancer [for example stimulation of autophagy and apoptosis (Carracedo et al., 2006b; Guzman et al., 2006; Salazar et al., 2009), inhibition of cell proliferation (Guzman et al., 2006), decreased VEGF signaling (Blazquez et al., 2004) and MMP-2 down-regulation (Blazquez et al., 2008)]., These encouraging findings fostered the interest on the utilization of cannabinoids in cancer therapies. However, they also underlined the need for additional preclinical and clinical studies aimed at optimizing the use of cannabinoids (see Box 2).

In line with this idea and based on the observations described in the previous section showing that the combination of THC, CBD and TMZ enhances the anticancer activity of each of these antineoplastic agents (Scott et al., 2014; Torres et al., 2011), a Phase 1/2 clinical study in recurrent GBM patients is being conducted to assess the safety and effectiveness of the administration of the cannabinoid-based medicine Sativex concomitantly with TMZ (NCT01812603 and NCT01812616). A high percentage of newly diagnosed GBM presents innate resistance to TMZ (Mrugala, 2013). This resistance has been related with several molecular alterations, including the methylation of the methylguanine-DNA methyltransferase (MGMT) promoter (Hegi et al., 2005). Preclinical data support that the combination of cannabinoids and TMZ exerts a strong anticancer action even when MGMT is over-expressed (Torres et al., 2011) thereby suggesting that this type of therapy might potentially help to improve the overall response to TMZ treatment in glioblastoma.

Synthetic cannabinoids are also being evaluated in clinical studies. For example, *dexanabinol*, [an enantiomer HU-210 (a mixed CB1/CB2 cannabinoid receptor agonist) which does not bind with significant affinity to cannabinoid receptors but instead acts a NMDA receptor antagonist (Feigenbaum et al., 1989)], is currently undergoing Phase 1 trials for the treatment of brain cancer and advanced solid tumors (NCT01489826).

7. Conclusions and future directions

Despite the existence of conflicting reports relative to the role of the endocannabinoid system in cancer generation and progression and several reports pointing to a possible tumor-promoting immunosuppressive role of cannabinoids (Cudaback et al., 2010; Hart et al., 2004; McKallip et al., 2005; Zhu et al., 2000) a large body of scientific evidences strongly support THC and other cannabinoid agonists exert anticancer actions in preclinical models of cancer (including immunocompetent mice) through a well-established mechanism of action. There is also a good evidence that cannabinoids enhance the anticancer activity of TMZ and ALK inhibitors in animal models of glioma. These observations provide preclinical proof-of-concept that cannabinoids could enhance the efficacy of classical cytotoxic drugs at least in glioblastoma (Fig. 1). However, additional studies are required to analyze the efficacy of these drug combinations in other cancer types as well as to identify additional cannabinoid-based drug combinations that could be useful for the treatment of glioma or other types of cancer. Likewise, further research is required to identify the precise molecular cross-talk mechanisms that become activated upon exposure of cancer cells to cannabinoids in combination with different chemotherapeutic agents.

Regarding patient stratification, one important step forward would be to identify which patients are potentially responsive to cannabinoid treatment. To this aim, it would be desirable that future clinical trials aimed at analyzing the anticancer activity of cannabinoid-based medicines would include translational studies in which specific biomarkers associated to a better or worse response to cannabinoid treatment could be identified.

In conclusion there exist solid scientific evidences supporting that cannabinoids exhibit a remarkable anticancer activity in preclinical models of cancer. Since these agents also show an acceptable safety profile, clinical studies aimed at testing them as single agents or in combinational therapies are urgently needed. Results from these studies are essential to clarify whether cannabinoids (and specifically cannabinoid-based medicines) could be helpful in the fight of cancer.

Potential conflict of interest

We declare that GW Pharmaceuticals funded part of the research of our laboratory. Likewise, part of the data obtained by the authors in relation with the antitumor action of cannabinoids is included in three patent applications presented by GW Pharmaceuticals.

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