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OPINION

Towards the use of cannabinoids as antitumour agents

Guillermo Velasco, Cristina Sánchez and Manuel Guzmán

Abstract | Various reports have shown that cannabinoids (the active components of marijuana and their derivatives) can reduce tumour growth and progression in animal models of cancer, in addition to their well-known palliative effects on some cancer-associated symptoms. This Opinion article discusses our current understanding of cannabinoids as antitumour agents, focusing on recent insights into the molecular mechanisms of action, including emerging resistance mechanisms and opportunities for combination therapy approaches. Such knowledge is required for the optimization of preclinical cannabinoid-based therapies and for the preliminary clinical testing that is currently underway.

Few plant species have been the subject of so much scientific, clinical and social debate as *Cannabis sativa* L. (marijuana). Preparations from this plant have been used for many centuries both medicinally and recreationally. However, the chemical structures of their unique active components — the cannabinoids — were not elucidated until the 1960s. Three decades later, the first solid clues on cannabinoid molecular action were established, which led to an impressive expansion of basic cannabinoid research and to a renaissance in the study of the therapeutic effects of cannabinoids in various fields, including oncology.

Today, it is widely accepted that, of the ~70 cannabinoids produced by *C. sativa*, Δ^9-tetrahydrocannabinol (THC) is the most relevant owing to its high potency and abundance in plant preparations[1,2]. THC exerts a wide variety of biological effects by mimicking endogenous substances — the so-called endocannabinoids (the two most studied being anandamide[3] and 2-arachidonoylglycerol (2-AG)[4,5]) — that engage specific cell-surface cannabinoid receptors[6] (FIG. 1).

So far, two major cannabinoid-specific receptors — CB1 and CB2 — have been cloned and characterized from mammalian tissues[7,8]. In addition, other receptors, including the transient receptor potential cation channel subfamily V member 1 (TRPV1) and certain orphan G protein-coupled receptors, GPR55, GPR119 and GPR18, have been proposed to act as endocannabinoid receptors[6]. Most of the effects that are produced by cannabinoids in the nervous system and in non-neural tissues rely on CB1 receptor activation. Expression of this receptor is abundant in the central nervous system, particularly in discrete areas that are involved in the control of motor behaviour (such as the basal ganglia and cerebellum), memory and learning (the cortex and hippocampus), emotions (the amygdala), sensory perception (the thalamus), and autonomic and endocrine functions (the hypothalamus, pons and medulla). In addition, CB1 receptors are expressed in peripheral nerve terminals and in many extra-neural sites. By contrast, the CB2 receptor was initially described as present in the immune system[6], but more recently it has also been shown to be expressed in additional cell types[9,10]. Notably, expression of CB1 and CB2 receptors has been found in many types of cancer cells, although this does not necessarily correlate with the expression of these receptors in the tissue type of origin[9,11,12].

The endocannabinoids, together with their receptors and the proteins that are responsible for their synthesis, transport and degradation, constitute the endocannabinoid system. Aside from its pivotal neuromodulatory activity[13] (FIG. 1), the endocannabinoid system exerts other regulatory functions in the body, such as the control of cardiovascular tone, energy metabolism, immunity and reproduction[14,15]. This miscellaneous activity makes the pharmacological manipulation of the endocannabinoid system a promising strategy for the management of many different diseases. Specifically, cannabinoids are well-known to exert palliative effects in cancer patients[14,15], and their best-established use is in the inhibition of chemotherapy-induced nausea and vomiting[15,16]. Today, capsules of THC, named dronabinol (Marinol; Solvay Pharmaceuticals), and its synthetic analogue

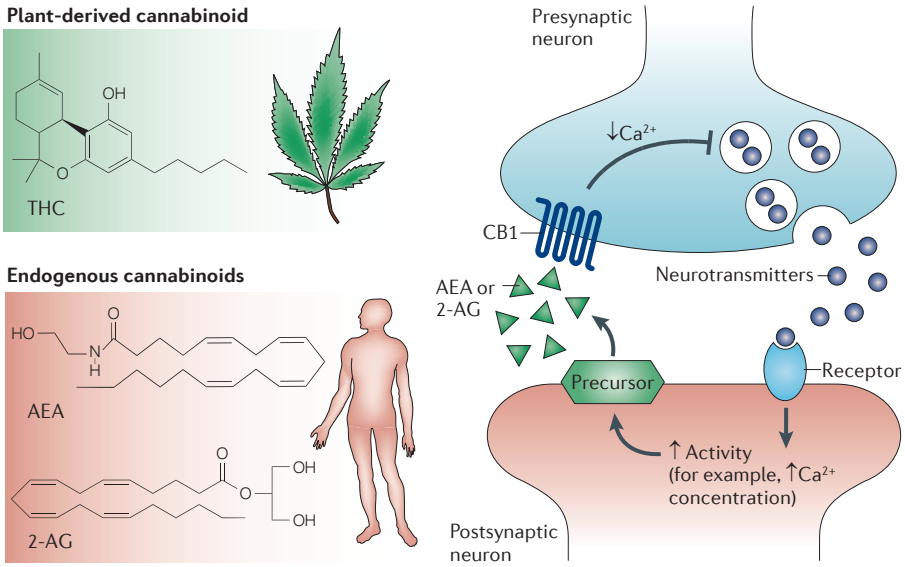

Figure 1 | **Cannabinoids and endocannabinoids.** Plant-derived cannabinoids such as Δ^9-tetrahydrocannabinol (THC) function in the body by activating specific cannabinoid receptors that are normally engaged by a family of endogenous ligands — the endocannabinoids anandamide (*N*-arachidonoylethanolamine (AEA))[3] and 2-arachidonoylglycerol (2-AG)[4,5]. A well-established function of the endocannabinoid system is its role in neuromodulation. After the binding of neurotransmitters to their receptors, activated postsynaptic neurons synthesize membrane-bound endocannabinoid precursors and cleave them to release endocannabinoids[13]. This is generally induced by an increase in the cytosolic concentration of free Ca^{2+} (REF. 13). Endocannabinoids subsequently act as retrograde messengers by binding to presynaptic CB1 cannabinoid receptors, which are coupled to the inhibition of Ca^{2+} influx into the cell and, in turn, to the blockade of neurotransmitter release[13]. This allows the tuning of key biological processes such as memory, movement, appetite and pain[14]. Figure is modified, with permission, from REF. 16 © (2003) Macmillan Publishers Ltd. All rights reserved.

nabilone (Cesamet; Meda Pharmaceuticals), are approved for this purpose. Cannabinoids also inhibit pain, and thus a standardized cannabis extract, nabiximols (Sativex; GW Pharmaceuticals) has been approved in Canada and is currently the subject of large-scale Phase III clinical trials for managing cancer-associated pain. Another potential palliative effect of cannabinoids in oncology, which is supported by Phase III clinical trials, includes appetite stimulation and attenuation of wasting. In relation to this, dronabinol can currently be prescribed for anorexia that is associated with weight loss in patients with AIDS.

The therapeutic potential of cannabinoids in oncology might not be restricted to their aforementioned palliative actions. Thus, numerous studies carried out during the past few years have provided evidence showing that THC and other cannabinoids exhibit antitumour effects on a wide range of animal models of cancer[12,16] (Supplementary information S1 (table)). Moreover, these observations led to the development of a pilot clinical study to investigate the antitumour activity of THC in patients with glioma. Nonetheless, a few studies have shown that, under certain conditions, cannabinoid treatment can stimulate cancer cell proliferation *in vitro*[17,18] and can interfere with the tumour-suppressor role of the immune system[19,20]. Likewise, there have been conflicting reports regarding the role (tumour suppressor or oncogenic) of the endocannabinoid system in cancer[21].

In this Opinion article we summarize these observations and provide an integrated view of the molecular mechanisms that are responsible for cannabinoid anti-tumour activity. Likewise, we discuss the experimental evidence that supports the existence of mechanisms of resistance to the cell death-promoting actions of THC in certain types of cancer cells, as well as the possible strategies that could be undertaken to overcome such resistance. We also discuss the preclinical data that support the potential combined administration of cannabinoids and other drugs in anticancer therapies.

The endocannabinoid system and cancer

Little is currently known about the biological role of the endocannabinoid system in cancer physiopathology. Although there are some exceptions that may be tumour type-specific, both cannabinoid receptors and their endogenous ligands are generally upregulated in tumour tissue compared with non-tumour tissue[16,21–23]. Additionally, different studies have associated the expression levels of cannabinoid receptors, endocannabioids and endocannabinoid-metabolizing enzymes with tumour aggressiveness[21,24,25], which suggests that the endocannabinoid system may be over-activated in cancer and so it may be pro-tumorigenic[21]. In support of this, in mouse models of cancer, genetic ablation of CB1 and CB2 receptors reduces ultraviolet light-induced skin carcinogenesis[26], and CB2 receptor overexpression enhances predisposition to leukaemia following leukaemia virus infection[27].

Conversely, and in line with evidence that the pharmacological activation of cannabinoid receptors reduces tumour growth[12,16], the upregulation of endocannabinoid-degrading enzymes has been observed in aggressive human tumours and cancer cell lines[24,25], indicating that endocannabinoid signalling can also have a tumour-suppressive role. In support of this, the deletion of CB1 receptors accelerates intestinal tumour growth in a genetic mouse model of colon cancer[28]; increased endocannabinoid levels diminish azoxymethane-induced precancerous lesions in the mouse colon[29]; and a reduction in the expression of the endocannabinoid-degrading enzyme monoacylglycerol lipase reduces tumour growth in xenografted mice[24].

Therefore, further studies — including those involving the genetic or pharmacological manipulation of the endocannabinoid system — will be required to dissect the precise signalling mechanisms that regulate cannabinoid-induced cell death or cell proliferation. Such information is needed to clarify the contextual determinants that determine when this system acts as a guardian, or, alternatively, as an inducer of tumorigenesis or tumour progression.

Preclinical antitumour activity

Since the late 1990s, a large body of evidence has accumulated demonstrating that various cannabinoids exert antitumour effects in a wide variety of experimental models of cancer, ranging from cancer cell lines in culture to genetically engineered mice (Supplementary information S1 (table)). Multiple cannabinoids have shown this activity, including THC; the endocannabinoids 2-AG and anandamide; and different synthetic cannabinoid receptor agonists that have either comparable affinity for CB1 and CB2 receptors (for example, WIN 55,212-2 and HU-210), higher affinity for CB1 (for example, methanandamide) or higher affinity for CB2 (for example, JWH-133). These findings strongly support

the suggestion that, aside from the role of the endogenous cannabinoid system in cancer, pharmacological stimulation of CB receptors is, in most cases, antitumorigenic. Nonetheless, a few reports have proposed a tumour-promoting effect of cannabinoids[17–20]. These apparently conflicting observations are discussed below.

Cannabinoids impair tumour progression at different levels. Their most prevalent effect is the induction of cancer cell death by apoptosis and the inhibition of cancer cell proliferation (FIG. 2). At least one of these actions has been demonstrated in almost all the cancer cell types tested (TABLE 1; see Supplementary information S1 (table)). In addition, *in vivo* experiments have shown that cannabinoids impair tumour angiogenesis and block invasion and metastasis (FIG. 2).

Mechanisms of antitumour effects

Induction of cancer cell death. A considerable amount of the research that has been conducted so far on the mechanism of cannabinoid antitumour activity has focused on glioma cells. Initial studies showed that THC and other cannabinoids induce the apoptotic death of glioma cells through CB1- and CB2-dependent stimulation of the *de novo* synthesis of the pro-apoptotic sphingolipid ceramide[23,30–32].

Further studies, based on the analysis of the gene expression profiles of THC-sensitive and THC-resistant glioma cells, gave further insight into the specific signalling events downstream of ceramide that are activated in cancer cells by cannabinoids[33]. THC acutely upregulates the expression of the stress-regulated protein p8 (also known as NUPR1), which is a transcriptional regulator that has been implicated in the control of tumorigenesis and tumour progression[34], together with several of its downstream targets such as the endoplasmic reticulum (ER) stress-related transcription factors ATF4 and CHOP (also known as DDIT3), as well as the pseudokinase tribbles-homologue 3 (TRIB3)[33] (FIG. 3).

The ER stress response is a complex intracellular signalling pathway that becomes activated in response to Ca^{2+} depletion, oxidative injury, a high-fat diet, hypoglycaemia, viral infections and exposure to certain anticancer agents[35]. ER stress aims to lessen the protein load on the ER by coordinating a temporal shutdown in protein translation and a complex programme of gene transcription to increase the ER protein-folding capacity. If this transcriptional programme fails to re-establish proper ER homeostasis, persistence in ER stress can induce cell death, usually by activating intrinsic apoptosis[35]. ER stress, as induced by different anticancer agents, can also lead, through different mechanisms[36], to the stimulation of autophagy, which is an essential cellular process that participates in a number of physiological functions in the cell[36,37].

During autophagy, organelles and other cytoplasmic components are engulfed in double-membrane vesicles that are designated autophagosomes. The maturation of these vesicles involves their fusion with lysosomes, which in turn leads to the degradation of the autophagosome components by lysosomal enzymes[37]. Autophagy is primarily a cytoprotective mechanism, although its activation can also lead to cell death[37,38]. Indeed, THC-triggered stimulation of the p8-regulated pathway enhances the inhibitory interaction of TRIB3 with a pro-survival kinase, AKT, which leads to the inhibition of the mammalian target of rapamycin complex 1 (mTORC1) and the subsequent stimulation of autophagy-mediated cell death[39] (FIG. 3). Cannabinoids induce autophagy in different types of cancer cells in culture (TABLE 1), and pharmacological or genetic inhibition of autophagy prevents cannabinoid antitumour action in different animal models of cancer (FIG. 3), thus demonstrating that autophagy is important for cannabinoid antineoplastic activity[39,40]. Moreover, autophagy blockade prevents cannabinoid-induced apoptosis and cell death, whereas apoptosis blockade prevents cannabinoid-induced cell death but not autophagy[39,40]. This indicates that autophagy is upstream of apoptosis in the mechanism of cannabinoid-induced cell death (FIG. 3).

The direct participation of the p8-mediated autophagy pathway in the antitumour action of cannabinoids has been clearly demonstrated in glioma cells, as well as in pancreatic and hepatic cancer cells[33,39–41]. At least part of this signalling route has also been found to be upregulated on cannabinoid treatment in other types of cancer cells (TABLE 1). This suggests that — with some variations — this could be a general mechanism by which the activation of CB1 and CB2 receptors promotes cancer cell death.

Additional mechanisms may nonetheless cooperate with the p8-mediated autophagy pathway to evoke cancer cell death (FIG. 3). For example, in hepatocellular carcinoma cells, cannabinoids can trigger an ER stress-dependent activation of AMPK that cooperates with the TRIB3-mediated inhibition of the AKT–mTORC1 axis in the stimulation of autophagy-mediated cell death[40]. In melanoma[42], breast carcinoma[22] and prostate carcinoma[43] cells cannabinoids can induce cell cycle arrest together with apoptosis[22,42,43]. Notably, cannabinoid antiproliferative

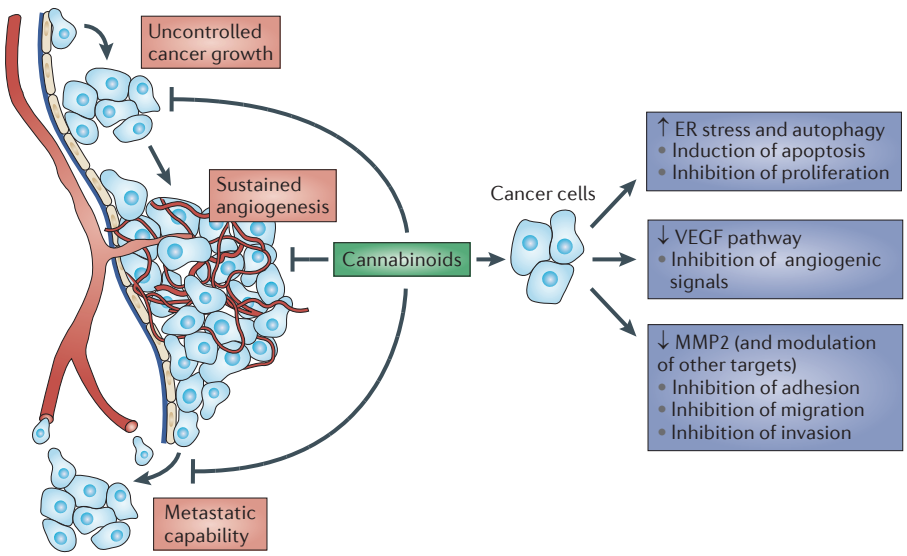

Figure 2 | **General mechanisms of cannabinoid antitumour action.** Cannabinoids block tumour progression by targeting several hallmarks of cancer. They impair uncontrolled cancer cell growth by inducing cancer cell death by apoptosis[23,31,39,41] and by inhibiting cancer cell proliferation[22,42,85]. They also hamper tumour angiogenesis by downregulating the vascular endothelial growth factor (VEGF) pathway in cancer cells[30,49–51]. Finally, cannabinoids hinder metastasis by inhibiting cancer cell adhesion and migration/invasiveness through the modulation of matrix metalloproteinase 2 (MMP2), tissue inhibitor of matrix metalloproteinases 1 (TIMP1), inhibitor of DNA binding 1 (ID1) and perhaps other targets[53–57,59]. ER, endoplasmic reticulum.

Table 1 | **Cannabinoids activate a similar pro-apoptotic mechanism in different types of cancer cells***

Cancer cell	CB receptor involved	Ceramide synthesis	ER stress	p8–TRIB3 induction	AKT inhibition	Autophagy	Apoptosis	Refs
Glioma	CB1 and CB2	✓	✓	✓	✓	✓	✓	39
Pancreatic cancer	CB2	✓	✓	✓	✓	✓	✓	39,41
Hepatocellular carcinoma	CB2	✓	✓	✓	✓	✓	✓	40
Breast cancer	CB2	ND	ND	✓	✓	✓ (UO)‡	✓	94
Rhabdomyosarcoma	CB1	ND	✓	✓	✓	ND	✓	95
Mantle cell lymphoma	CB1 and CB2	✓	✓	ND	ND	✓ (WIN 55,212-2)§	✓(WIN 55,212-2)§	96
Leukaemia	CB2	✓	ND	ND	✓	ND	✓	86,97,98
Prostate cancer	CB2	✓	ND	ND	✓	ND	✓	99,100
Melanoma	CB2	ND	ND	✓	✓	✓ (UO)‡	✓	42
Lung carcinoma	ND	ND	ND	ND	✓	ND	✓	56

CB, cannabinoid; ER, endoplasmic reticulum; ND, not determined; TRIB3, tribbles-homologue 3; UO, unpublished observations. *The existence of experimental evidence for the participation of CB receptors, *de novo*-synthesized ceramide, ER stress induction, upregulation of p8 and/or of TRIB3, autophagy induction or apoptosis in cannabinoid-induced death for each type of cancer cell is indicated by a tick. ‡G.V., C.S. and M.G., unpublished observations. §WIN 55,212-2 produces a cytoplasmic vacuolization (autophagic-like) phenotype in mantle cell lymphoma, an effect that seems to be CB receptor-independent.

action — at least in melanoma[42] and breast cancer[22] cells — also relies on AKT inhibition.

Likewise, the effect of cannabinoids in hormone-dependent tumours may at least partly rely on their ability to interfere with the activation of growth factor receptors[12,16]. Some of these, as well as other mechanisms[44], may participate in the cytotoxic action of cannabinoids in different types of cancer cells together with the autophagy-mediated cell death pathway. However, further investigation is required to clarify this issue.

Notably, cannabidiol (CBD; a phytocannabinoid with a low affinity for cannabinoid receptors[15]) and other marijuana-derived cannabinoids[45] have also been proposed to promote the apoptotic death of cancer cells by acting independently of CB1 and CB2 receptors. The mechanism by which CBD produces this effect has not yet been completely clarified, but it seems to rely — at least in part — on its ability to enhance the production of reactive oxygen species in cancer cells[46–48].

Inhibition of angiogenesis, invasion and metastasis. In cancer cells, cannabinoids block the activation of the vascular endothelial growth factor (VEGF) pathway, which is an inducer of angiogenesis. Specifically, different elements of this cascade, such as the main ligand (VEGF) and the active forms of its main receptors (VEGFR1 and VEGFR2), are downregulated on cannabinoid treatment of skin carcinomas[49], gliomas[30,50] and thyroid carcinomas[51]. In vascular endothelial cells, cannabinoid receptor activation inhibits proliferation and migration, and induces apoptosis[50,52]. These and perhaps other cannabinoid-evoked actions result in a normalized tumour vasculature; that is, smaller and/or fewer vessels that are more differentiated and less leaky.

Likewise, cannabinoids reduce the formation of distant tumour masses in animal models of both induced and spontaneous metastasis (Supplementary information S1 (table)) and inhibit adhesion, migration and invasiveness of glioma[53], breast[54,55], lung[56,57] and cervical[57] cancer cells in culture. These effects depend, at least in part, on the modulation of extracellular proteases (such as matrix metalloproteinase 2 (MMP2))[53] and their inhibitors (such as tissue inhibitor of matrix metalloproteinases 1 (TIMP1))[57].

Notably, pharmacological inhibition of ceramide biosynthesis abrogates the anti-tumour and anti-angiogenic effect of cannabinoids in glioma xenografts, and decreases VEGF production by glioma cells *in vitro* and *in vivo*[30]. Likewise, inhibition of MMP2 expression and glioma cell invasion is prevented by blocking ceramide biosynthesis and by knocking down p8 expression[53]. Although further research is still necessary to precisely define the molecular mechanisms that are responsible for these actions of cannabinoids, these observations indicate that the ceramide and p8-regulated pathway has a general role in the antitumour activity of cannabinoids that target CB1 and CB2 receptors.

It is worth noting that CBD, by acting independently of CB1 and CB2 receptors, produces an antitumour effect — including, the reduction of invasiveness and mestastasis — in different animal models of cancer (Supplementary information S1 (table)). This effect of CBD seems to at least partly rely on the downregulation of the helix–loop–helix transcription factor inhibitor of DNA binding 1 (ID1)[58,59].

Cannabinoid selectivity for cancer cells. Although research conducted during the past few years has shed light on the intracellular signalling mechanisms that underlie cannabinoid anticancer action, several important observations — particularly those related to the role of cannabinoid receptors in triggering these signals — remain to be clarified. Thus, the viability of normal (non-transformed) cells is unaffected or, under certain conditions, even favoured by cannabinoid challenge[31–33,39,60]. For example, THC treatment of astrocytes (a cell type that expresses functional CB1 receptors) does not trigger the activation of ER stress, the upregulation of the p8 pathway, the inhibition of the AKT–mTORC1 axis or the stimulation of autophagy and apoptosis, even when concentrations of THC that are higher than those that promote glioma cell death are used[33,39]. Similar results were obtained with primary embryonic fibroblasts[33,39] and other types of non-transformed cells expressing functional cannabinoid receptors when compared with their transformed counterparts[22,42,49,61]. Nonetheless, certain populations of non-transformed cells, particularly those that are highly proliferative, such as endothelial vascular cells in culture, undergo apoptosis and cell death in response to THC treatment[50]. Likewise, selective

pharmacological stimulation of CB2 receptors can induce the apoptosis of different types of immune cells, a mechanism which has been proposed to be involved in the immunosuppressive actions of cannabinoids[62,63] (discussed below). In any case, even considering these exceptions, the stimulation of cannabinoid receptors seems to be coupled to the activation of different signalling mechanisms in transformed and non-transformed cells. The precise molecular reasons for this different behaviour remain important open questions in cannabinoid research that need to be clarified.

Another intriguing observation is that, in some types of cancer cells, such as glioma cells, pharmacological blockade of either CB1 or CB2 receptors prevents cannabinoid-induced cell death with similar efficacy[31,64], but in other types of cancer cells (for example, pancreatic[41], breast[22] or hepatic[40] carcinoma cells) antagonists of CB2 but not of CB1 receptors inhibit cannabinoid anti-tumour actions. It is not yet known why cannabinoids produce their antitumour actions through one or the other receptor type depending on the type of cancer cell studied.

Notably, the stimulation of cannabinoid receptors may lead to important changes in the processes that regulate antitumour immunity. Thus, for example, the treatment of mice with THC triggers a shift in their cytokine profile — from T helper 1 (T_H1) to T_H2 (REFS 19,65–67) — and induces the mobilization of myeloid-derived suppressor cells[68]. These two events have a crucial role in the suppression of antitumour immunity. In agreement with this notion, the stimulation of CB2 receptors has been proposed in some reports to enhance tumorigenesis by interfering with tumour surveillance by the immune system[19,20]. By contrast, cannabinoids may also enhance immune-mediated tumour surveillance in some contexts: the antitumour action of WIN 55,212-2 (a CB1 and CB2 mixed agonist) or JWH-133 (a CB2-selective agonist) was more pronounced in melanoma xenografts that were grown in immunocompetent mice compared with those grown in immunodeficient mice[42]. This also indicates that, at least in this model, stimulation of CB2 receptors primarily inhibits tumour growth through direct effects on cancer cells rather than necessarily through interfering with the normal antitumour function of the immune system. In line with this idea, treatment of immunocompetent rats for 2 years with very high doses of THC (50 mg per kg per day, five times per week) decreased the incidence of several types of tumours and increased the overall survival of these animals[61]. These observations might be related to the ability of THC to reduce inflammation (a pro-tumorigenic function of the immune system)[69,70], and this effect that may be beneficial for preventing certain types of cancer[70]. For cannabinoid use to be clinically successful, antitumour effects will need to overcome immunosupresive (potentially

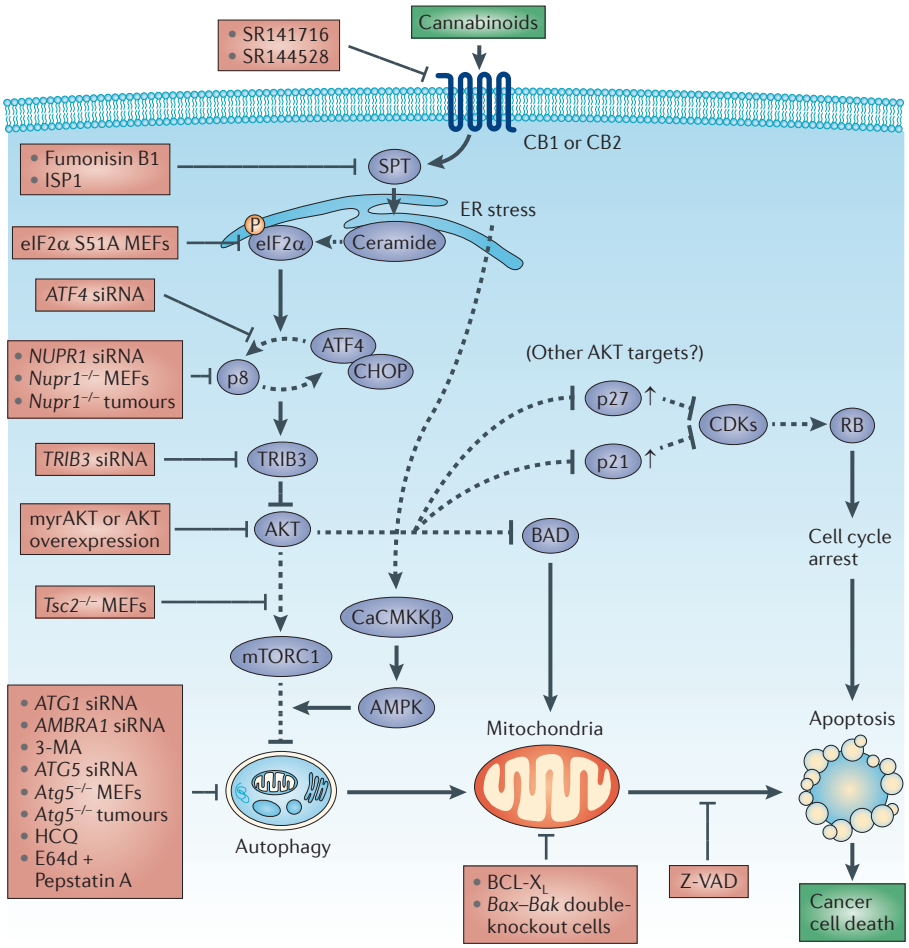

Figure 3 | **Cannabinoid-induced apoptosis relies on the stimulation of ER stress and autophagy.** The figure depicts the cumulative understanding of the mechanisms of cannabinoid-induced apoptosis from studies in glioma, pancreatic and hepatocellular carcinoma cells. These signalling routes may constitute the main mechanisms of cannabinoid-induced cell death, with some variations being inherent to different types of cancer cells. Cannabinoid agonists bind to CB1 and/or CB2 receptors to stimulate *de novo* synthesis of ceramide[31–33,39,86], which triggers the induction of an endoplasmic reticulum (ER) stress-related response that promotes the upregulation of the transcription factor p8 and several of its downstream targets, including the pseudokinase tribbles-homologue 3 (TRIB3)[33,39]. This favours the interaction of TRIB3 with AKT[39,87], thus leading to the inhibition of the AKT–mammalian target of rapamycin complex 1 (mTORC1) axis, and the subsequent induction of autophagy[39]. Autophagy is upstream of intrinsic mitochondrial apoptosis in the process of cannabinoid-induced cell death. The importance of this pathway is highlighted by the ability of different chemical and genetic manipulations (shown in red boxes) to block cannabinoid-induced cell death. In hepatocellular carcinoma cells the cannabinoid-evoked and ER stress-dependent activation of calcium/calmodulin-dependent protein kinase kinase-β (CaCMKKβ; also known as CAMKK2) and AMP-activated protein kinase (AMPK) leads, together with the p8–TRIB3 pathway, to autophagy and apoptosis[40]. The cannabinoid-evoked inhibition of AKT could promote cycle arrest in breast cancer and melanoma cells, as well as apoptosis, through additional mechanisms, including the decreased phosphorylation (P) of the pro-apoptotic protein BAD[88], and the activation of the cyclin-dependent kinase (CDK) inhibitory proteins p21 and p27 (REFS 22,42,89). This would lead to the subsequent decreased phosphorylation of RB, which thus would be active to arrest the cell cycle. 3-MA, 3-methyladenine; ATG, autophagy-related; eIF2α, eukaryotic translation initiation factor 2A; HCQ, hydroxychloroquine; ISP1, serine palmitoyltransferase inhibitor (also known as thermozymocidin); MEF, mouse embryonic fibroblast; myrAKT, myristoylated AKT; *NUPR1*, nuclear protein transcriptional regulator 1; siRNA, small interfering RNA; SPT, serine palmitoyltransferase, *Tsc2*, tuberous sclerosis 2.

tumour-promoting) effects. Additional studies should clarify this issue. For example, it could be conceivable to study the effect of cannabinoid administration on the generation and progression of tumours that exhibit different sensitivity to cannabinoids and that are grown in immunocompetent or immunodeficient mice in which the expression of CB1 and/or CB2 receptors in cells from the immune system has been genetically manipulated.

Finally, some cannabinoid receptor agonists promote cancer cell death more efficiently than other agonists that exhibit similar or even higher affinity for CB1 or CB2 receptors. For example, THC promotes cancer cell death in a CB1- and/or a CB2-dependent manner at lower concentrations than the synthetic cannabinoid receptor ligand WIN 55,212-2 (REF. 41) (G.V., C.S. and M.G., unpublished observations), although WIN 55,212-2 displays significantly higher affinity for CB1 and CB2 receptors in binding assays[6].

Further work that aims to investigate, for example, CB receptor homo-oligomerization or hetero-oligomerization in response to different cannabinoid agonists[71], their association with specific domains in the plasma membrane such as lipid rafts[72], changes in the subcellular localization of CB receptors, and the selective coupling to different G proteins and other signalling proteins[73] will be essential to precisely define the role of each cannabinoid receptor type in anticancer signalling.

Resistance mechanisms

Numerous studies have contributed to our appreciation of the heterogeneity of cancer, whereby each subtype of cancer, and even each individual tumour, exhibits a series of molecular characteristics that determines its behaviour and, in particular, its responsiveness to different anticancer drugs. In agreement with this line of reasoning, a recent report investigated the molecular features that are associated with the resistance of a collection of human glioma cell lines and primary cultures to cannabinoid antitumour action[64].

The study showed that, although the apoptotic effect of THC on glioma cells relied on the stimulation of CB receptors and the activation of the p8-mediated autophagy pathway, the differences in sensitivity to THC-induced cell death correlated with the enhanced expression of a particular set of genes in the THC-resistant glioma cells rather than with the presence of different expression levels of CB1 or CB2 receptors[64]. Interestingly, upregulation of one of these genes, midkine (*MDK*), which encodes a growth factor that has previously been associated with increased malignancy and resistance to anticancer therapies in several types of tumours[74,75], correlates with a lower overall survival of patients with glioblastoma[64]. Moreover, MDK has a direct role in the resistance to THC action via stimulation of anaplastic lymphoma kinase (ALK)[76] (FIG. 4). Thus, the stimulation of ALK by MDK inhibits the THC-evoked autophagy-mediated cell death pathway. Further research should clarify whether this mechanism could also be responsible for the resistance of cancer cells that express high levels of MDK to other therapies. Interestingly, *in vivo* silencing of MDK or pharmacological inhibition of ALK in a mouse xenograft model abolishes the resistance to THC treatment of established tumours derived from cannabinoid-resistant glioma cells[64].

Taken together, these findings support the idea that stimulation of the MDK–ALK axis promotes resistance to THC antitumour action in gliomas and could help to set the basis for the potential clinical use of THC in combination with inhibitors of this axis (FIG. 4). In line with this idea, ALK inhibitors have started to be used in clinical trials for the management of non-small-cell lung cancer and other types of tumours[77,78]. Future research should clarify whether this mechanism of resistance to cannabinoid action operates in other types of tumours. In agreement with this possibility, MDK silencing enhanced the sensitivity of cannabinoid-resistant pancreatic cancer cells to THC-induced cell death[64].

The release of other growth factors by cancer cells has also been implicated in the mechanism of resistance to cannabinoid

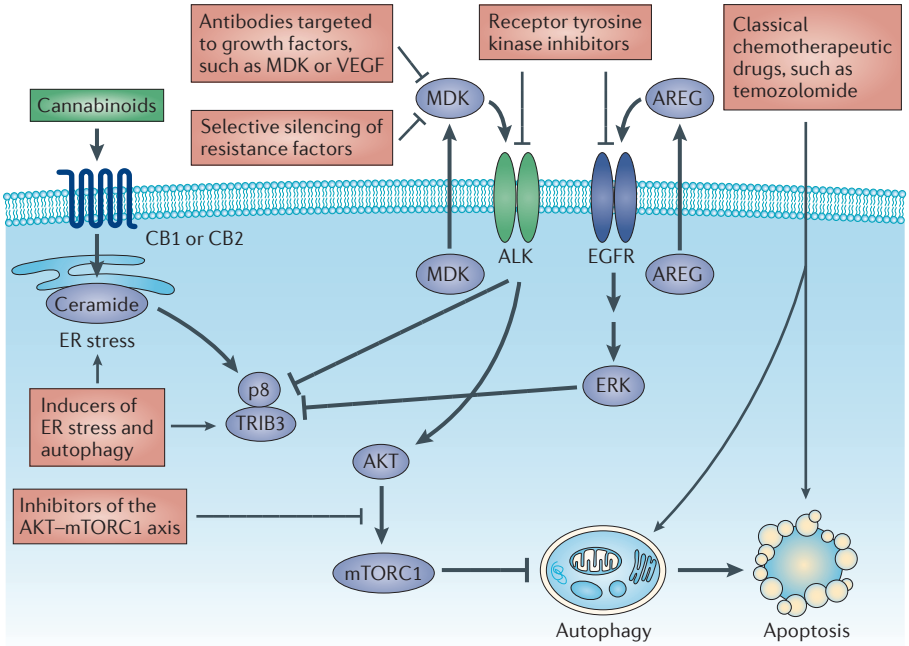

Figure 4 | **Possible strategies that aim to optimize cannabinoid-based therapies against gliomas.** Glioblastoma is highly resistant to current anticancer therapies[90–92]. Specifically, the resistance of glioma cells to cannabinoid-induced cell death relies, at least in part, on the enhanced expression of the growth factor midkine (MDK) and the subsequent activation of the tyrosine kinase receptor ALK[64]. Likewise, enhanced expression of the heparin-bound EGFR-ligand amphiregulin (AREG) can promote resistance to Δ^9-tetrahydrocannabinol (THC) antitumour action via ERK stimulation[79]. Combination of THC with pharmacological inhibitors of ALK (or genetic inhibition of MDK) enhances cannabinoid action in resistant tumours, which provides the rationale for the design of targeted therapies that are capable of increasing cannabinoid antineoplastic activity[64]. Combinations of cannabinoids with classical chemotherapeutic drugs such as the alkylating agent temozolomide (TMZ; the benchmark agent for the management of glioblastoma[90,93]) have been shown to produce strong anticancer action in animal models[80]. Combining cannabinoids and TMZ is thus an attractive possibility for clinical studies that aim to investigate cannabinoid antitumour effects in glioblastoma. Other potentially interesting strategies to enhance cannabinoid anticancer action (still requiring additional experimental support in preclinical models) could be combining cannabinoids with endoplasmic reticulum (ER) stress and/or autophagy inducers or with inhibitors of the AKT–mammalian target of rapamycin complex 1 (mTORC1) axis. VEGF, vascular endothelial growth factor.

antitumour action. Thus, increased expression of the heparin-bound epidermal growth factor receptor (EGFR) ligand amphiregulin is associated with enhanced resistance to THC antitumour action in glioma xenografts[79]. Notably, and illustrating that the dose of cannabinoids could be crucial for their optimal therapeutic effect, low (submicromolar) concentrations of THC or other synthetic cannabinoid agonists enhance the proliferation of several cancer cell lines *in vitro*. This effect relies on the activation of the protease ADAM17, the shedding of heparin-bound EGFR ligands, including amphiregulin, and the subsequent stimulation of ERK and AKT pathways[18]. In line with this idea, a recent report has shown that treatment with the synthetic cannabinoid CP-55,940 increases the proliferation of murine glioma cells that were engineered to express CB1 or CB2 receptors only when these receptors were coupled to AKT activation[17]. Although a protumorigenic effect has not been observed on the growth of tumour xenografts of glioma cells and treated with low doses of THC[80] (Supplementary information S1 (table)), increased expression of amphiregulin promotes resistance to THC antitumour action through a mechanism that involves the EGFR-dependent stimulation of ERK, and the subsequent inhibition of p8 and TRIB3 expression. Likewise, pharmacological inhibition of EGFR, ERK[79] or AKT (G.V., C.S. and M.G, unpublished observations) enhances the cell death-promoting action of THC in cultures of glioma cells. These observations suggest that targeting EGFR, and the AKT and ERK pathways could enhance the antitumour effect of cannabinoids.

Combinational therapies

The use of combinational anticancer therapies has several theoretical advantages over single-agent-based strategies, as they allow the simultaneous targeting of tumour growth, progression and spreading at different levels. In line with this idea, recent observations support the hypothesis that the combined administration of cannabinoids with other anticancer drugs acts synergistically to reduce tumour growth. For example, the administration of THC and temozolomide (the benchmark agent for the management of glioblastoma) exerts a strong antitumour action in glioma xenografts, an effect that is also evident in temozolomide-resistant tumours[80]. Interestingly, no toxicity was observed in mice treated with combinations of THC and temozolomide[80]. As most patients with glioblastoma undergo temozolomide treatment, these findings indicate that the combined administration of temozolomide and cannabinoids could be therapeutically exploited for the management of glioblastoma (FIG. 4).

Likewise, another study has recently shown that the combined administration of gemcitabine (the benchmark agent for the treatment of pancreatic cancer) and different cannabinoid agonists synergistically reduces the viability of pancreatic cancer cells[81]. Other reports indicate that anandamide and HU-210 may also enhance the anticancer activity of paclitaxel[82] and 5-fluorouracil[83], respectively.

An additional approach has been to combine THC with CBD. This combination enhances anticancer activity compared with THC alone and reduces the doses of THC that are needed to inhibit tumour growth[80,84]. Moreover, the combination of THC and CBD together with temozolomide produces a striking reduction in the growth of glioma xenografts even when low doses of THC are used[80]. Of note, CBD has also been shown to alleviate some of the undesired effects of THC administration, such as convulsions, discoordination and psychotic events, and, therefore, improves the tolerability of cannabis-based medicines[15]. As mentioned above, *C. sativa* produces ~70 different cannabinoids and, apart from CBD, some of the other cannabinoids present in marijuana might attenuate the psychoactive side effects of THC or might even produce other therapeutic benefits[15]. Thus, we think that clinical studies that aim to analyse the efficacy of cannabinoids as antitumour agents should be based on both the use of pure substances, such as THC and CBD, and the use of cannabis extracts containing controlled amounts of THC, CBD and other cannabinoids.

Clinical antitumour effects

Although the clinical approval of cannabinoids is mostly restricted to palliative uses in various diseases, following promising preclinical data, the antitumour effects of cannabinoids are beginning to be clinically assessed. In a pilot Phase I clinical study, nine patients with actively growing recurrent glioblastoma who had previously failed standard therapy underwent intracranial THC administration[11]. Under these conditions, cannabinoid delivery was safe and could be achieved without substantial unwanted effects. In addition, although no significant conclusions can be extracted from a cohort of nine patients, the results obtained in that study suggested that some patients responded — at least partially — to THC treatment in terms of decreased tumour growth rate, as evaluated by magnetic resonance imaging[11]. Importantly, analyses of samples obtained from two patients in the study before and after THC administration indicated that the molecular mechanism of cannabinoid antitumour action discussed above (p8 and TRIB3 upregulation[33,39], mTORC1 inhibition[39], stimulation of autophagy and apoptosis[11,33,39], inhibition of cell proliferation[11], decreased VEGF signalling[30] and MMP2 downregulation[53]) also occurs in cancer patients. These findings were encouraging, and they reinforced the interest in the potential use of cannabinoids in cancer therapies. However, they also highlighted the need for further research that aims to optimize the use of cannabinoids in terms of patient selection, combinations with other anticancer agents and the use of other routes of administration. The route of administration requires careful consideration. The most widely used route of administration of recreational and self-medicated marijuana is smoking. Although THC and other phytocannabinoids are rapidly absorbed by inhalation, smoking is an unattractive clinical option. Although preclinical work in animal models has typically administered cannabinoids intratumourally, our work indicates that systemic (oral or intraperitoneal) administration of cannabinoids effectively reduces tumour growth (G.V., C.S. and M.G., unpublished observations). Therefore, we propose that future clinical studies to determine the efficacy of cannabinoids as antitumour agents should be based on oral or oro-mucosal treatments.

Conclusions and future directions

It is widely believed that strategies that aim to reduce mortality from cancer should consist of targeted therapies that are capable of providing the most efficacious and selective treatment for each individual tumour and patient. Thus, the major focus of anticancer drug development has moved progressively from non-specific chemotherapies to molecularly targeted inhibitors. However, despite the huge amount of preclinical literature on how these rationally designed compounds work, the advance in the use of most of these drugs in clinical practice is still limited. Thus, for 'personalized' cancer therapy to gain clinical impact, stronger efforts should be made on both preclinical, hypothesis-testing grounds (for example, unambiguous identification of molecular targets and therapeutic windows of experimental therapies) and patient-stratification procedures (for example, the identification and assessment of predictive biomarkers). The application of these principles has provided beneficial

returns to patients in other clinical settings, as exemplified by the development of inhibitors of oncogenic protein kinases such as BCR–ABL (for chronic myeloid leukaemia), KIT and PDGFR (for gastrointestinal stromal tumours), and BRAF (for melanoma)[77].

How do cannabinoid-based medicines fit into this ongoing scenario? Let us consider gliomas, the type of cancer on which the most detailed cannabinoid research has been conducted to date. As discussed above, the engagement of a molecular target (such as CB receptors) by a family of selective drugs (such as THC and other cannabinoid agonists) inhibits tumour growth in animal models through a well-established mechanism of action that seems to be active in patients. Moreover, cannabinoids potentiate the antitumour efficacy of temozolomide and ALK inhibitors in mice that harbour gliomas. These findings provide preclinical proof-of-concept that 'cannabinoid sensitizers' could improve the clinical efficacy of classical cytotoxic drugs in glioblastoma (FIG. 4), and perhaps other highly malignant tumours such as pancreatic cancer, melanoma and hepatocellular carcinoma. However, further research is required to define the precise molecular crosstalk between cannabinoids and chemotherapeutic drugs and to optimize the pharmacology of preclinical cannabinoid-based combinational therapies to maximize antitumour efficacy without unacceptable toxicities to normal tissues.

Regarding patient stratification, we should unequivocally determine which particular individuals are potentially responsive to cannabinoid administration. For this purpose, high-throughput approaches should be implemented to find cannabinoid therapy-associated biomarkers in tumour biopsies or, ideally, in easily acquired fluids containing circulating cancer cells or enhanced levels of resistance factors that could have been released by cancer cells. These biomarkers would conceivably relate to cannabinoid pharmacodynamics — namely, the expression and activity of CB receptors and their downstream cell death-inducing effectors. This would be analogous to the biochemical evaluation of oestrogen and ERBB2 receptors, which predict the benefit from endocrine therapies and trastuzumab, respectively, in breast cancer. Predictive markers to define the sensitivity of a particular tumour to cannabinoid-based therapies could also include the status of growth factors (such as MDK in gliomas), as well as their receptors and signalling partners.

In conclusion, cannabinoids induce tumour cell death and inhibit tumour angiogenesis and invasion in animal models of cancer, and there are indications that they also do so in patients with glioblastoma. As cannabinoids show an acceptable safety profile, clinical trials testing them as single drugs or, ideally, in combination therapies in glioblastoma and other types of cancer are feasible and promptly needed.

Guillermo Velasco, Cristina Sánchez and Manuel Guzmán are at the Department of Biochemistry and Molecular Biology I, School of Biology, Complutense University, and the Instituto Universitario de Investigación Neuroquímica (IUIN), Madrid 28040, Spain.

Guillermo Velasco and Manuel Guzmán are also at the Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid 28040, Spain.

Guillermo Velasco is also at the Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Madrid 28040, Spain

Manuel Guzmán is also at the Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid 28040, Spain.

Correspondence to G.V.
e-mail: gvd@bbm1.ucm.es

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Competing interests statement

The authors declare competing financial interests. See Web version for details.

FURTHER INFORMATION

Cannabinoid Signalling Group:
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SUPPLEMENTARY INFORMATION

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