New uses warranted for cannabinoids? Recent data indicate that synthetic or endogenous substances activating the receptor for marijuana's psychotropic component might be used as templates for the development of new anti-cancer drugs.

Targeting the endocannabinoid system in cancer therapy: A call for further research

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After almost four millennia of more-or-less licit recreational and medicinal use of *Cannabis sativa*, the nature of the principle psychotropic constituent of this renowned plant (–)-Δ^9-tetrahydrocannabinol (THC), was elucidated between the 1940s and 1960s (refs. 1,2). This breakthrough eventually opened the way to the identification first of the sites of action of THC, the cannabinoid CB_1 and CB_2 receptors[3], and subsequently of the endocannabinoids, endogenous agonists of the cannabinoid receptors[4]. CB_1 receptors are expressed in several brain regions, with high concentrations in the basal ganglia, hippocampus, cerebellum and cortex, and mediate the typical psychotropic effects of *Cannabis*, marijuana and THC. Lower, albeit functionally active, amounts of CB_1 receptors are also found in several peripheral tissues and cell lines, whereas CB_2 receptors are mostly confined to immune tissues and cells and seem to underlie the immune-suppressant actions of THC (ref. 4). Both CB_1 and CB_2 receptors are expressed by cells from the early stages of fertilized oocyte development[5], and CB_1 expression in the developing brain is substantially different from that observed in the adult brain[6]. These observations, together with the ubiquity of the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) in both vertebrate and invertebrate tissues, and their modulatory activity on proteins and nuclear factors involved in cell proliferation, differentiation and apoptosis, suggest that the endocannabinoid signaling system might be involved in the control of cell survival, transformation or death[7].

The anti-neoplastic activity of THC and its analogs was first observed in the early 1970s (ref. 8), before the discovery of cannabinoid receptors and endocannabinoids. Surprisingly, although these observations were of potential interest, no in-depth investigations were performed on this topic at the time. This contrasts with the investigation of the therapeutic effects of cannabinoids on some cancer-related disorders, such as emesis and nausea. Indeed, the only therapeutic uses for which oral THC (Dronabinol, Marinol) and its synthetic derivative nabilone (Cesamet) have received regulatory approval in the United States are the alleviation of nausea and emesis for cancer patients undergoing chemotherapy and the stimulation of appetite for patients with AIDS. Although the clinical efficacy of these palliative effects of THC is now being debated[9,10], recent studies have revisited the possibility that drugs targeting the endogenous cannabinoid system might also be used to retard or block cancer growth.

Cannabinoids and solid tumor growth

Based on the immunosuppressive effects of *Cannabis*, studies were originally performed in animals to investigate the possibility that marijuana smoking, or long-term THC treatment, might favor tumor growth. These studies, however, produced contradictory results. For example, the data of one study suggested that the growth of a lung carcinoma was enhanced[11], whereas in a two-year chronic administration study with high THC doses, a reduction of the spontaneous onset of hormone-dependent tumors occurred[12]. Another *in vivo* study demonstrated that both marijuana and placebo smoke result in the suppression of the growth of sarcoma 180 tumors[13]. A parsimonious interpretation of these investigations is that, although some pro-tumor effects of THC are due to CB_2 receptor–mediated immune suppression[11], marijuana smoke, like tobacco or cocaine smoke, might favor the onset of lung cancer by causing bronchial epithelium damage[14].

Other experiments have been undertaken to determine the effect of endogenous cannabinoid receptor ligands on cancer cells *in vitro*. It was found that 4–5-day treatment of human breast cancer cell (HBCC) lines with sub-micromolar concentrations of endocannabinoids results in complete blockade of cell proliferation[15]. This effect is mediated by the CB_1 receptor subtype and is due to inhibition of the action of endogenous prolactin, which HBCCs in culture use as an autocrine growth factor. In fact, anandamide inhibits the expression of prolactin receptors in these cells[15], and agents activating the CB_1 receptor, via the same mechanism, also counteract the proliferation of human prostate cancer cells induced by exogenous prolactin[16]. Indeed, both human breast and prostate cancer cells were shown to express high levels of CB_1 receptors that had never been detected previously in the corresponding healthy tissues. HBCCs also respond to the nerve growth factor (NGF) by proliferating more rapidly, and two-day treatment of HBCCs with CB_1 receptor agonists suppresses the levels of *trk* proteins, the receptors for NGF, thereby resulting in the inhibition of NGF-induced proliferation[16]. Thus, endocannabinoids seem to act as selective inhibitors of growth factor–dependent breast and prostate cancer-cell proliferation (Fig. 1). It is possible that, by interfering with the expression of other growth and mitogenic factors, substances that activate CB_1 receptors might also exert more general anti-tumor as well as anti-angiogenic effects.

Inhibition of proliferation, however, is not the only mechanism through which cannabinoid receptor agonists block solid tumor growth *in vitro* (Table 1, Fig. 1). THC was found to induce apoptosis of glioma and prostate cancer cells, even though the involvement of cannabinoid receptors in these early studies was unclear[17,18]; more recently, a similar effect by anandamide was suggested to be mediated by another controversial target for this compound, the vanilloid VR1 receptor[19,20].

Most of the compounds that inhibit the growth of cancer cells *in vitro* turn out to be disappointingly ineffective when tested in animals. However, there is now evidence that substances that activate cannabinoid receptors may act as anti-

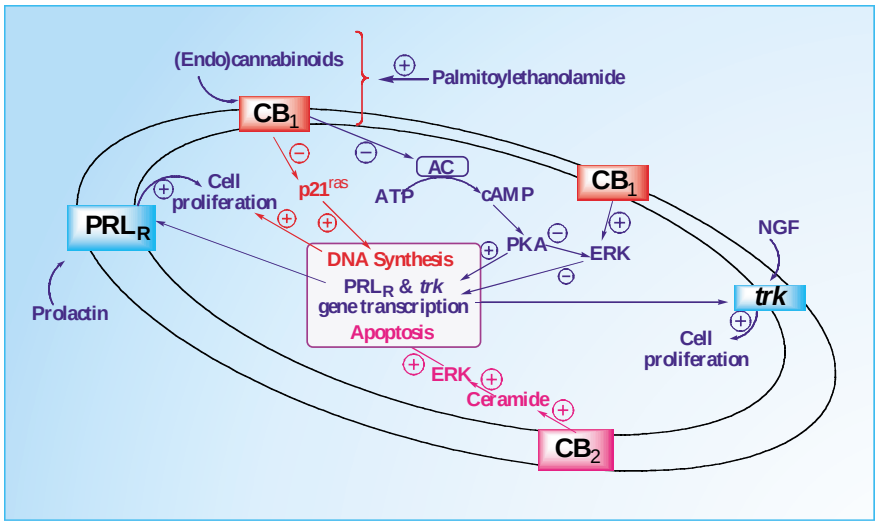

Fig. 1 Intracellular pathways underlying the anti-tumor effects of cannabinoids. Activation of cannabinoid CB_1 receptors in human breast and prostate cancer cells leads to modulation of cell proliferation by inhibiting the expression at the transcriptional level of the receptors (PRL_R and *trk*, respectively) of prolactin and NGF. These effects are due to inhibition of protein kinase A (PKA) signaling (via inhibition of adenylyl cyclase (AC) and of cAMP formation) and to either relief of inhibition or direct stimulation of ERK (refs. 7,26). In transformed epithelial thyroid cells, CB_1 activation inhibits the activity of the K-*ras* oncogene product, $p21^{ras}$, thereby leading to the inhibition of the *ras* cascade and of cell division[23]. Palmitoylethanolamide enhances the anti-proliferative effect of anandamide and, to a minor extent, of synthetic cannabinoids[29]. Cannabinoid CB_2 receptor stimulation, by acting via sustained synthesis of ceramide and activation of ERK, triggers nuclear events leading to the programmed death (apoptosis) of glioma cells[21,22].

neoplastic drugs *in vivo*. Intratumoral THC administration can effectively reduce the growth of gliomas in mice by inducing apoptosis of the tumor cells. This effect is attenuated by a combination of cannabinoid CB_1 and CB_2 receptor antagonists[21]. More recently, it was reported that selective activation of the cannabinoid CB_2 receptor results in a striking inhibition of glioma growth *in vivo*, and that CB_2 receptor expression correlates with the level of malignancy in astrocytomas[22]. These studies, which relate to a type of malignant tumor for which a successful treatment has yet to be developed, have resulted recently in the unprecedented decision by the Spanish government to allow a clinical study aimed at investigating the effect of intra-tumoral THC administration on glioma in humans.

Repeated intra-tumoral administration of a low, non-psychotropic dose of a metabolically stable anandamide analog, met-fluoro-anandamide, inhibits the growth of tumors induced in nude mice by injection of rat thyroid epithelial FRTL-5 cells transformed into cancer cells by the oncogene K-*ras*[23]. This anti-tumor effect is almost entirely erased by a selective antagonist of CB_1 receptors, which, accordingly, are found in tumors derived from transformed thyroid cells. Moreover, this effect is accompanied by a strong reduction of the activity of the K-*ras* oncogene protein product, $p21^{ras}$, and is due, as in the case of HBCCs (ref. 15), to blockade of the cell cycle prior to the entry into the DNA synthetic (S) phase. So, once again, interference with a mitogenic signal underlies a cytostatic action by a cannabinoid CB_1 receptor agonist (Fig. 1). It was also shown that the expression of CB_1 receptors is oppositely regulated in healthy and transformed thyroid cells (as well as in tumors derived from these latter cells) following treatment with met-fluoro-anandamide, and is suppressed or enhanced in healthy or cancer cells, respectively. Thus, the degree of CB_1 receptor expression determines the extent of the responsiveness of normal or transformed FRTL-5 cells to (endo)cannabinoids[23].

The enhancement of cannabinoid receptor expression in malignant versus healthy tissues, observed so far in gliomas and transformed thyroid cells[22,23], might suggest a possible role of the endocannabinoid system in the tonic suppression of cancer growth. However, other than the finding of alterations of anandamide and/or 2-AG levels in some tumors as compared with the corresponding healthy tissues[24,25], no evidence has been reported thus far to support this hypothesis.

Progress has been made instead towards the understanding of the intracellular events underlying the *in vitro* and *in vivo* anti-tumor effects of cannabinoid receptor agonists. It is now established that THC and endocannabinoids stimulate the activity of proteins that are downstream of the activation of $p21^{ras}$, that is, the mitogen-activated protein kinases (MAPKs)[7]. In HBCCs, inhibition of extracellular signal-regulated kinase (ERK), a particular class of MAPKs, counteracts the effects of anandamide on cell proliferation and on prolactin and NGF receptor expression[26]. The apoptotic effect of THC on glioma cells seems to be mediated by sustained ceramide synthesis and ERK-dependent pathways[21,22]. Hence, it is possible that by modulating the activity of both $p21^{ras}$ and MAPKs, the cannabinoid receptors regulate the fate of cancer cells (for example, apoptosis or cessation of proliferation) (Fig. 1).

Can cancer therapies target the endocannabinoid system?

The findings discussed here, in our opinion, should prompt further studies on the therapeutic potential in cancer treatment of substances that modulate the activity of cannabinoid receptors or the levels of endocannabinoids, particularly as other possible targets for the anticancer action of these compounds, such as metastasis and angiogenesis, are still largely unexplored. Cannabinoids appear to be well tolerated in animal studies and do not produce the generalized toxic effects in normal tissues that limit most conventional agents used in chemotherapy. However, together with obvious social, political and legal considerations, the therapeutic application of agonists selective for CB_1 receptors, as in treatments for breast and thyroid cancer, should be weighed against the undesired psychotropic side effects expected from the stimulation of these receptors in the brain. Although the activation of 'central' CB_1 receptors has been and still is currently exploited to alleviate two typical symptoms of cancer patients under chemotherapy, that is, lack of appetite and nausea, other psychotropic effects that are likely to follow from chronic treatment with cannabinoids, such as attentional dysfunction and impairment of cognitive and psychomotor performance[27], might be poorly tolerated. Furthermore, the potential for ad-

diction and tolerance to psychoactive cannabinoids after prolonged use has not been yet fully assessed. On the other hand, the administration of compounds selective for the CB_2 receptor, as in the treatment of gliomas, would be devoid of psychotropic effects but might cause the immune-suppressive effects typical of plant cannabinoids, which seem to be mediated mostly by this receptor subtype and, at least in one case, have been reported to counteract the immune defense against tumor growth[11].

In those scenarios where effectiveness against cancer-cell growth were to be conclusively proven *in vivo*, the side effects of CB_1-selective agonists might be overcome, at least in principle, by using one or a combination of the following strategies also listed in Table 1: 1) intra-tumoral application of cannabinoids seems to result in little, if any, undesired 'central' effects in mice[21–23], although its safety and efficacy in humans still needs to be assessed; 2) the use of these substances in combination with non-psychotropic 'entourage' compounds, which lower the threshold of concentrations necessary to observe CB_1 receptor-mediated tumor suppressing effects *in vitro*[28,29], should also be investigated *in vivo*; 3) partial agonists of cannabinoid receptors that are also capable of activating vanilloid receptors, such as the synthetic compound *arvanil*[30], inhibit cancer-cell growth *in vitro* more potently and efficaciously than 'pure' agonists of either receptor type[20,30] (these compounds are likely to have a lower addictive potential than full agonists of CB_1 receptors, and might be used as templates for the development of new, potent multi-target anticancer agents to be tested *in vivo*); and 4) CB_1 receptor agonists that do not cross the blood–brain barrier (BBB) should be developed and evaluated against cancer cell growth.

Future research should also address the question of whether or not endogenous cannabinoids exert tumor-suppressing effects, as such a discovery might result in another approach for the development of possibly harmless anti-cancer drugs. In fact, selective inhibitors of endocannabinoid degradation with no direct action on CB_1 receptors, even if administered systemically, would exhibit little if any psychotropic activity and be most effective only in those tissues where the levels of endocannabinoids are pathologically altered.

In conclusion, only further efforts towards the full assessment of the effects of substances selectively targeting the endocannabinoid system will provide the answer as to whether these compounds might be exploited successfully as novel anticancer agents. The recent findings discussed here indicate that more basic and clinical research is needed not only to understand if cannabinoids are as effective and safe as other therapeutic drugs in the palliative care of cancer[9,10], but also if they

Table 1 Applications, mechanisms and pros and cons of cannabimimetics in cancer therapy

	CB_1 agonists	CB_2 agonists	Endocannabinoids (anandamide, 2-AG)	Inhibitors of endocannabinoid degradation	CB_1/vanilloid receptor 'hybrid' agonists
Possible application	Mammary, prostate and thyroid carcinoma, neuroblastoma, glioma	Glioma	Mammary, prostate, and thyroid carcinoma	Mammary carcinoma	Mammary and prostate carcinoma, neuroblastoma, glioma
Mechanism of action	Inhibition of the mitogen-induced stimulation of the G_0/G_1-S phase of the cell cycle	Induction of apoptosis	Inhibition of the mitogen-induced stimulation of the G_0/G_1-S phase of the cell cycle	Increase of the possible tonic inhibition of cancer cell growth exerted by endocannabinoids	Inhibition of the mitogen-induced stimulation of the G_0/G_1-S phase of the cell cycle; induction of apoptosis
Intracellular signals	Inhibition of PKA; inhibition of $p21^{ras}$ activity; activation of ERK	Sustained stimulation of ceramide; activation of ERK	Inhibition of PKA; inhibition of $p21^{ras}$ activity; activation of ERK	Signals normally induced by endocannabinoids	Inhibition of PKA; activation of ERK; strong increase in $[Ca^{2+}]_i$
Advantages	Little or no toxicity; little or no suppression of the immune response	No psychotropic or addictive effects; little or no toxicity	Little or no toxicity; little or no suppression of the immune response (with anandamide)	Little or no toxicity; little or no suppression of the immune response; no dependence; possible simultaneous stimulation of appetite and attenuation of emesis and pain	High potency; little or no suppression of the immune response; no pungency
Disadvantages	Induction of psychotropic effects; possible induction of dependence	Interference with the immune response	Little efficacy due to metabolic instability	Effective only if there is a tonic inhibition of cancer-cell growth by endocannabinoids	Toxicity not assessed yet
Ways to circumvent the disadvantages	Intra-tumor administration; co-administration of 'entourage' compounds; use of analogs that do not cross the BBB	Unknown	Use of metabolically stable analogs	None	Thorough assessment of toxicity
Possible future developments	Inhibition of metastasis and/or angiogenesis?	Unknown	Inhibition of metastasis and/or angiogenesis?	Unknown	Unknown

Speculations on the possible future developments of the use of endocannabinoid-based drugs in cancer therapy is based on unpublished data from our laboratories and on the increasingly high number of reports of endocannabinoid effects on the activity of protein kinases and nuclear factors involved in cancer-cell focal adhesion and migration[3,4,7]. The use of inhibitors of endocannabinoid degradation must still be regarded as purely hypothetical and is subordinated to the finding of endocannabinoids tonically inhibiting tumor growth *in vivo*.

can be used to retard tumor growth and spreading instead of, or in addition to, conventional chemotherapy agents.

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